UNIVERSITY OF SOUTHAMPTON

Assessment of Organically-Bound Tritium (OBT) Dispersion and Accumulation in the Environment.

Krista E. Doucette

This Thesis is submitted for the Degree of Master of Philosophy

October 2002

FACULTY OF SCIENCE

DEPARTMENT OF CHEMISTRY

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF SCIENCE

CHEMISTRY

Master of Philosophy

ASSESSMENT OF ORGANICALLY-BOUND TRITIUM (OBT) DISPERSION AND ACCUMULATION IN THE ENVIRONMENT

Krista Doucette

Measurements of tritium (^3H) in fish in the Cardiff Bay area have consistently shown levels greater than the surrounding seawater as well as having concentrations significantly higher than those found in fish from other areas of the British Isles. The elevated concentrations of this radionuclide in the Severn Estuary arise from the authorised discharges of wastes from Hinkley point and the laboratories at Nycomed –Amersham. The wastes that these establishments discharge differ significantly in that Hinkley discharges ^3H predominantly in the form of tritiated water whilst Nycomed- Amersham waste is a mixture of tritiated water and uncharacterised ^3H-labelled organic compounds.

A combustion method was developed for the analysis of total ^3H in sediment samples, with a limit of detection of 0.01- 0.03 Bq/g. The total ^3H concentrations of sediments analysed in the area were in the order of 0.2- 0.5 Bq/g, with highest activities found in areas surrounding the sewer pipeline near Nycomed-Amersham. Seasonal reductions in sediment ^3H levels were found to result from extreme storm and tidal activity, linked to re-suspension of the fine surface sediments during the winter months. The tritium found in the sediments was associated with clay rich particulate matter and in the form of organically bound tritium (OBT), where OBT was measured as the difference between total tritium and water-extractable tritium. The nature and characteristics of the OBT was investigated using a series of solvent extraction systems covering a range of polarities. Failure of the solvents to extract more than ~30% of the activity, with values near to the limit of detection, makes it difficult to characterise the nature of the OBT. This suggests that the OBT might be present in organisms which do not lyse during this extraction or that it is part of a complex molecule which needs more vigorous techniques to extract it from the sediment.

Contents

Page

1.Introduction to Study

1.3 The Severn Estuary

2. Tritium Facts and Figures

3. Tritium Measurements

3.3 Liquid Scintillation Counting

3.5. Total Tritium

4. Tritium in Cardiff Bay

5. Gamma Analysis of Samples

5.2 Results and Discussion

5.3 Normalisation of Data

5.4 Be-7 Activity

6. Partitioning Experiments

6.3 Partitioning experiments with standards in seawater

6.4 Partioning experiments with minerals and clays

6.5 Partitioning of OBT between water and organic solvents

7. Solvent extraction of sediment samples

7.1 Introduction

7.2 Soxhlet extractions of sediments

7.3 Sediment samples

7.5 Acid-digestion of Sediment Samples

8. Overall Conclusions

9.Appendices

List of Figures

List of Tables

Acknowledgements

Firstly, many thanks to Dr Alan Howard, Dr Ian Croudace and Dr Phill Warwick for their supervision and guidance during my time at the University of Southampton. Thanks also to my family and friends for their endless support and faith in the fact that I would eventually finish!

And to the FSA (RP0193) for their financial support, without which this project would not have been possible.

1. Introduction to Study

1.1Background to study

Measurements of tritium (^{3}H) in fish in the Cardiff area have consistently shown levels approximately one thousand times greater than the concentration in seawater, as well as having significantly greater concentrations than are found elsewhere in UK coastal waters.[CEFAS C0984, MAFF &SEPA 1999; Pearce 1999] Of this tritium, more than 90% is in the form of organically bound tritium (OBT), where OBT is defined as tritium which is bound to a carbon atom and is only released during decomposition, or in the presence of strong acids, bases or catalysts. [CEFAS C0984]

There are several explanations for these observed tritium levels. Laboratories in Cardiff, currently Nycomed-Amersham plc, discharge tritium in liquid waste. On the opposite side of the Bristol channel a nuclear power plant at Hinkley inlet discharges similar amounts of tritium. Preliminary data however indicate that the levels of tritium in fish caught close to Hinkley are lower than the levels in fish from the Cardiff area, by an order of magnitude. The total seawater tritium concentrations at both sites are however very similar. There are two main differences between the Cardiff site and the other. Nycomed-Amersham, unlike most other sites, does not discharge tritium in the form of tritiated water alone; its waste contains both tritiated water and a large range of organic compounds, some of which are tritiated. The liquid waste from the Cardiff site is also discharged into the Severn estuary via a 10km long pipeline, at other sites the discharge is more directly into the recipient water body. The effluent discharged from the Cardiff Nycomed-Amersham plant into the Severn is essentially untreated, with the sewer pipes passing through Cardiff into the Severn Estuary. [CEFAS C0984]

In May 1998, following a discussion on the levels of tritium being found in flounder, the EA (Environment Agency) requested that the company reduce liquid discharges into the Severn estuary. The requirement was to reduce the total activity discharged from the site and did not involve consideration of any particular chemical form of the tritium. In order to implement the reduction in a short timescale the lower volume, higher activity wastes, predominantly from the intermediate manufacturing processes, were retained on site. Since May 1998, the discharge of tritium liquid discharge has been reduced by around

80%. Withheld waste is currently stored on site and will be treated and recycled when a discharge reduction program is implemented.

1.2 Objectives of the study

In light of the nature of the liquid discharge from the Nycomed-Amersham plant, which is essentially untreated, it is important to try and understand the processes by which the tritium has become incorporated into the biota in the surrounding area and to assess the environmental impact. The overall aim of this study is to investigate the characteristics of the tritium in the Cardiff Bay area and to try and understand the processes and mechanisms behind its incorporation into the sediment. The main objectives of this study are outlined below;

- To establish a working definition for Organically-Bound tritium, non-aqueous tritium and tritiated water in the context of environmental processes, and to develop analytical protocols to distinguish between these various forms of tritium.
- To determine the association of tritium between the various components of the sediment found in the area, and to assess the exchangeability of tritium between the sediment and the seawater.
- To determine the nature and characteristics of the tritium found on the sediments and explanations for any seasonality or variation in measurements.

An understanding of the characteristics and nature of the Cardiff-Bay area are imperative to this study, as there are a number of factors which may determine the residence time of tritium in the area and the nature of the tritium associated with the sediment. A background on the Severn Estuary is outlined in Section 1.3.

1.3 The Severn Estuary

1.3.1 Geological setting

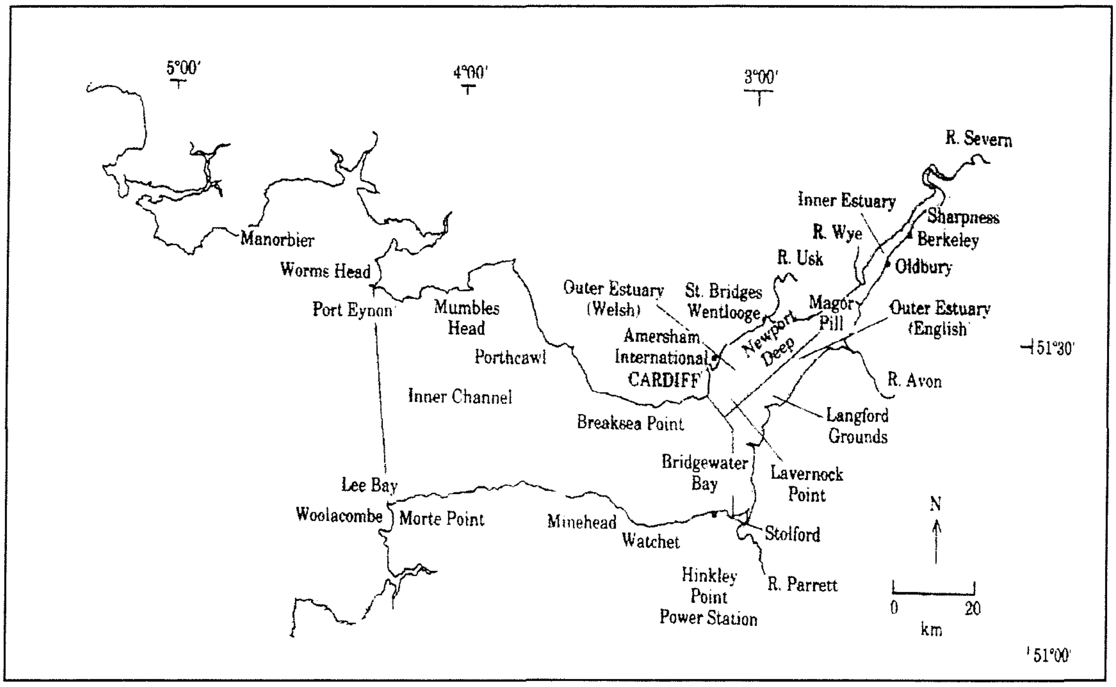


Figure 1.1: Severn Estuary and Bristol channel, modified from Halliwell & McKay 1994.

The Severn estuary, situated on the southwest coast of Britain as illustrated in Figure 1.1, is a large, flood dominated, partly rockbound system. It extends for 150km from Upton-on-Severn to Flat Holm and Steep Holm islands, before joining the Bristol Channel which extends westward from these islands to open into the Celtic sea.[Kirby & Parker 1983] It is one of the largest estuaries in the British Isles but, despite its size, it nevertheless displays, on its margins, substantial areas of now largely embanked estuarine alluvium. [Allen & Duffy, April 98] Although the rocky outcrops in places define the shore of the estuary, the margins are largely formed of broad intertidal mudflats, that in many instances border extensive areas of saltmarsh. [Allen 1987]

The Severn estuary is a large intertidal system, that has a temperate maritime climate, dominated by the easterly travel of the atmospheric depressions across the British Isles from the North Atlantic. [Chandler & Gregory 1976]. Due to its location, it faces the prevailing winds and is consequently swept by vigorous currents and is somewhat stormy. This has resulted in a system in delicate balance, which is likely to respond to modest changes in the tidal and/ or wind regimes. [Allen & Duffy, Sep 1998] It is an extremely macrotidal, well-mixed system that experiences a semidiurnal, flood dominated regime, containing in its upper intertidal zone, muddy sediments composed in bulk of interstratified quartzose silt- sand laminae and laminae dominated by clay minerals. [Allen 1988, Allen & Duffy, April 1998] The well-mixed system is typified by gradual salinity gradients and an extreme tidal range (14.8m) The chief forcing factors affecting the estuary are: relative sea level, the tidal regime and general windiness and the incidence of storms. [Allen & Duffy Sep 1998]

1.3.2 Tidal Regime

The Severn estuary has an extreme macrotidal, or hypertidal, predominantly semidiurnal regime, with marked diurnal and lunar-monthly inequalities and a flood dominance. [Allen & Duffy, April 98] The area has an extreme tidal range. At Avonmouth for example, the greatest predicted astronomical tide is 14.8 m (not reached every year); the mean spring tide is 12.3m and the mean range at neaps is 6.5m. [Allen 1987] Due to this extreme tidal range, the tidal streams even at neaps are powerful and turbulent, often in excess of 1m/s in many places. [Hamilton 1979, Crickmore 1982, Uncles 1984] The currents and tidal amplitudes found here are amongst the largest in the world, and are thought to be caused by the influence of up-channel reductions in width and depth on the tidal wave progressing from the Celtic sea.[Collins 1987]

This area experiences large semi-monthly, monthly and six monthly inequalities in the tidal regime, which indirectly has a substantial contribution to the reworking of muddy sediments in the upper parts of the intertidal zone. The tidal range between spring and neap high water is quite considerable. It is not uncommon for large areas of mud to be dessicated during neaps in the spring and summer months. Autumn and winter months on the other hand generally keep the mudflats well wetted and it is not uncommon for them to freeze. [Allen 1987] Tidal height has the greatest effect on the amount of sediment in an area and the amount deposited is positively correlated with tidal height. [Allen & Duffy, April 98] Although the tidal currents move water particles some considerable distance (10 to 22 km) during each of the ebb and flood phases of a tidal cycle [Shaw 1980], the net displacement of an individual particle is relatively small. [Imer, 1974]

1.3.3 Wind-Wave climate

The Severn estuary is typical of other intertidal estuaries in the sensitivity of its mudflats to wind-wave action. The estuary is however, open to the prevailing winds from the west and southwest, and these winds together with northeasterly and northerly blows, are funneled along it, with the aspect of the estuary ensuring that both banks are comparatively exposed. [Allen 1987, Allen & Duffy April 1998] The windiest seasons tend to be autumn and winter, with frequent gales and occasional full storms. [Allen 1987]

Erosion of the mudflats is positively correlated with windiness typically on a seasonal scale, with losses in the late autumn-winter-early spring and gains during the late spring-summer-early autumn. A single storm is known to remove up to 0.1m of sediment from the mudflat.[Allen & Duffy, April 1998] Tides and wind-generated waves are the dominant physical processes which affect water movement. They create currents that interact with the bathymetry and available sedimentary material. Tidal currents in this area are essentially rectilinear and are directed along the main axes of the channel. [Collins 1987]

1.3.4 Sediment transport and sources

In order to understand the sediment transport and sources in the area of study, we must first define what an estuary is. According to Allen [1991] an estuary is defined as 'an environment transitional between a river and a shelf sea'. Sedimentation processes in estuaries vary according to the supply and transport of fine grained and coarse grained material, and their response to the physics of water movement. [Collins 1987]

The Severn estuary and the Bristol Channel to which it is coupled, receive substantial amounts of fluvial sediment annually. [Parker & Kirby 1982, Collins 1983, 1987] The sediment is fine grained, clay-mineral dominated, which is readily suspended and transported in huge volumes in the vigorously stirred tidal waters. [Allen 1990] The turbid waters hold of the order of 1×10^7 t of sediment, the composition of which is shown in Table 1.1

Clay-Mineral	*Mean (%)*	*SD(%)*
illite	51.67	5.24
expandable	29.50	6.53
kaolinite	14.28	2.52
chlorite	4.56	3.75

Table 1.1: Severn flood plain clay-mineral content [Allen 1991]

The silty- sandy clays that make up the muddy sediments of the estuary tend to coarsen towards the head of the system. The finest deposits, which are silty clays with infrequent stripes of clean quartz silt, occur in the Wentlooge formation and the older Rumney beds. [Allen 1987] One of the sampling sites investigated in this study, Peterstone, is situated in this area.

In the estuary, muddy sediments are reworked not only on tidal and seasonal scales, but also on periods of decades to centuries. [Allen 1987] Fine sediment concentrations vary on an hourly and semidiurnal scale, as erosion, advection and settlement follow the changing tidal energy. The concentration also varies with changing energy during spring-neap and lunar-monthly cycles. Fine sediment concentrations in the area peak during the winter months, probably in response to seasonally changing windiness, river discharge, and water temperature. [Allen & Duffy, Sep 1998] There is however, little lateral uniformity amongst the laminae that may have accumulated on the mudflats, even in the more sheltered places. [Allen & Duffy, April 1998] The winter deposits on marshes and mudflats are sandier than summer ones and therefore should have a lower erosion threshold. [Allen & Duffy, Sep 1998]

There is much controversy over the origin of the Bristol channel/ Severn estuary mud deposits. This dates back even as long ago as 1883, when Sollas produced a detailed discussion on the subject, using arguments that have changed very little to this day. Sollas stated that "The Severn and its tributaries are not, except when flooded, very muddy rivers; the wash of the cliffs is not, as a rule excessive; the sea if it furnishes anything, certainly cannot furnish much; and yet the vast body of estuarine water that extends from Weston to Portishead is never otherwise than a sea of more or less diluted mud." [Sollas, 1883] Sollas was able to show that (1) the currents are too strong in the main estuary to allow much deposition of mud to occur; (2) the muds in the sub-estuaries receive most of the material from the main estuary during the flood, but deposition occurs on the ebb; (3) there must be considerable storage of suspended sediment derived from the whole variety of potential sources but " the accumulation is always being diminished by withdrawals seaward."

The Severn barrage report suggests that the rivers (Parret, Axe, Yeo, Avon and Usk), if not the entire source of the muds, are certainly instrumental in their deposition. Although depositional processes in this area are not fully understood, Sollas' ebb deposition of Severn estuary sediments does appear to fit findings of an apparent source for the muds found in this area. The report highlights a number of other possibilities for the deposition of mud in this area including increased potential for flocculation and subsequent deposition caused by mixing fresh water and the more saline water of the main estuary. Another possibility is that the slight increase of suspended sediment at the river mouths, in vertical vortices, causes rapid sedimentation of both the river and estuarine suspended sediment populations. Both of these processes however, can only occur at times of slack water.

1.3.4.1 Modeling of sediment transport

Sediment transport is difficult to model, especially in the case of estuaries, for a variety of reasons:

1. Water movements are tidally dependent, changing in their direction, intensity and duration.
2. Small errors in grain-size characteristics, bathymetry, bed type and bed friction can create significant errors in the model. [Black & Barnett 1998]

Despite these problems, a number of numerical modeling studies have been undertaken. It is however, generally recognised that it is difficult to realise their full potential because of a lack of calibration, verification of data and gaps in understanding of the fundamental sedimentation processes. Surficial mud for example, may not everywhere represent the present sedimentological regime. [Mclaren and Collins 1989]

1.4 References

Allen, J.R.L., *Reworking of muddy intertidal sediments in the Severn Estuary, Southwestern UK: a preliminary study,* Sedimentary Geology, **50**, p.1-23, 1987.

Allen, J.R.L., *Modern-period muddy sediments in the Severn Estuary (Southwestern UK): a pollutnat-based model for dating and correlation,* Sedimentary Geology, **58**, p.1-21, 1988.

Allen, J.R.L., *The Severn Estuary in southwest Britain: Its retreat under marine transgression, and fine-sediment regime,* Sedimentary Geology, **66**, p.13-28, 1990.

Allen, J.R.L., *Fine sediment and its sources, Severn Estuary and Inner Bristol Channel, Southwest Britain,* Sedimentary Geology, **75**, p.57-65, 1991.

Allen, J.R.L., Duffy, M.J., *Temporal and spatial depositional patterns in the Severn Estuary, Southwestern Britain: Intertidal studies at spring-neap and seasonal scales 1991-1993,* Marine Geology, **146**, p.147-171, Apr. 1998.

Allen, J.R.L., Duffy, M.J., *Medium-term sedimentation on high intertidal mudflats and salt marshes in the Severn estuary, SW Britain: The role of wind and ride,* Marine Geology, **150**, p.1-27, Sep. 1998.

Black , K.P., and Barnett, A.G., *Sediment tranport modeling under tidal flows with applications in a natural estuary;* IAHR Symposium on Mathematical Modelling of Sediment Transport in the Coastal Zone, Proceedings, Copenhagen, p.5-13, May 30-June 1, 1988.

CEFAS Contract tender C0984, *An assessment of the transfer of tritium through the marine foodchain,* For Nycomed-Amersham, Commercial in confidence.

Chandler, T.J., Gregory, S., The Climate of the British Isles, Longmann, London, p.390, 1976.

Collins, M.B., *Supply, distribution, and transport of suspended sediment in a macrotidal environment: Bristol Channel, UK.*, Canadian Journal of Fisheries and Aquatic Science, **40**, p.44-59, 1983.

Collins, M.B., *Sediment transport in the Bristol Channel: a review*, Proceedings of the Geological Association, **98**, p.367-383, 1987.

Crickmore, M.J., *Data collection-tides, tidal currents, suspended sediment.* In: Institution of Civil Engineers (Editor), Severn Barrage. Thomas Telford, London, p.19-26, 1982.

Haliwell, C.M., and McKay, W.A., *Application of a Simple Model to Predict Environmental Radionuclide Levels and Consequential Dose Rates on the south Welsh Coast, U.K.*, Estuarine, Coastal and shelf Science, **38**, p.207-216, 1994.

Hamilton, D., *The higher energy, sand and mud regime of the Severn Estuary, S.W. Britain.* In: R.T., Severn, D.L., Dineley and L.E., Hawker (Editors), Tidal Power and Estuary Management, Scientechnica, Bristol, p.162-172, 1979.

Imer, Institute for Marine Environmental Research (NERC) report 1973-1974, Imer, Plymouth, Devon, 71pp, 1974.

Kirby, R., and Parker, W.R., *Distribution and behaviour of fine sediment in the Severn Estuary and Inner Bristol Channel*, Canadian Journal of Fisheries and Aquatic Science, **40**(suppl. 1), p.83-95, 1983.

MAFF and SEPA (1999) Radioactivity in Food and the environment, 1998. RIFE-4. Ministry of Agriculture, Fisheries and Food and Scottish Environment Agency, London.

Mclaren, P., and Collins, M.B., *Sediment Transport Pathways in the Severn Estuary and Bristol Channel*, Severn Barrage Development Project, Document STPG/GEOSEA/3.1(iii)f, 54pp, 1989.

Parker, W.R., and Kirby, R., *Sources and transport patterns of sediment in the inner Bristol Channel and Severn Estuary,* In: Institution of Civil Engineers (Editors), Severn Barrage, Thomas Telford, London, p.181-194, 1982.

Pearce, J., *High tritium levels in Severn fish,* Marine Pollution Bulletin, **38**, p.3-4, 1999.

Shaw, T.L., *Physics of water movement in the Severn Estuary,* In: (Shaw, T.L.:ed), An environmental appraisal of tidal power stations: with particular reference to the Severn Barrage, Pitman, London, 220pp, 1980.

Sollas, W.J., *The estuaries of the Severn and its tributaries: an enquiry into the nature and origin of their tidal sediment and alluvial flats,* Quaterly Journal of the Geological Society, **39**, p.611-626, 1883.

Uncles, R.J., *Hydrodynamics of the Bristol Channel,* Marine Pollution Bulletin, **15**, p.47-53, 1984.

2. Tritium Facts and Figures

2.1 Tritium in the Environment

Tritium (^3H or T) is an isotope of hydrogen with a half-life of 12.35yrs.[NCRP 1985] Its atoms are three times heavier than hydrogen, resulting in an increase in both the specific gravity and boiling point of T_2O compared to water. [Okada and Momoshima 1993] It is a pure β emitter with a decay energy (Emax) of 18.6 keV, corresponding to maximum travelling distances in water and air of 6μm and 5mm respectively. [Warwick et al 1999; Belot et al 1996] Tritium decays with emission of an electron, with a mean energy (Eave) of 5.685 keV.[NCRP 1985]

Natural and anthropogenic sources contribute to the presence of tritium in the environment. The natural sources include its continuous production in the upper atmosphere (stratosphere), by the interaction of cosmic rays with the nuclei of the atmospheric gases, principally by neutron and proton induced reactions:

$^{14}N + n \rightarrow {}^3H + {}^{12}C$

$^{16}O + n \rightarrow {}^3H + {}^{14}N$

$^{14}N + p \rightarrow {}^3H + \text{products}$

$^{16}O + p \rightarrow {}^3H + \text{product}$

Once the tritium is released to the atmosphere it is rapidly oxidised to tritiated water HTO, on a time scale of 6-10 years. Nuclear reactions in the Earth's crust are other contributors.[Okada and Momoshima 1993; Etnier et al/ 1984, Craig and Lat 1961]

Anthropogenic sources include the nuclear weapons testing in the 1950s and '60s, continuous release from nuclear power plants and tritium production facilities under normal operations, as well as incidental releases from these facilities.[Okada and Momoshima 1993] There is very little exchange of tritium between the hemispheres; T injected into the northern hemisphere is found to a greater extent in the land and oceans of the northern hemisphere, very little can be detected in the southern hemisphere.[Belot et al 1996] In 1991 the inventory of naturally occurring tritium was estimated to be about $1x10^{18}$-$1.3x10^{18}$ Bq in the World, with typical ranges for background tritium concentrations at 0.02-0.5 Bq L^{-1} for surface seawater and 10-100 MBq m^{-3} for air. [Van Soy et al 1991; Ostlund 1974; Okai and Takashima 1991; Rozanski et al 1991a] During the period 1961-1963, just before the Test Ban Treaty of 1963, the release was most significantly estimated to be $1.7x10^{20}$ - $2.4x10^{20}$ Bq.[Kaufmann & Libby 1954; Buttlar & Libby 1955;United Nations 1977 &1982] The amount released from nuclear power plants and nuclear reprocessing plants is estimated to be $2x10^{16}$ Bq a^{-1}. [United Nations 1988] The predominant forms of tritium found in the environment are molecular tritium HT, tritiated water HTO, tritiated methane and organically bound tritium (OBT), with the majority being present in the form of HTO.[Hill and Johnson 1993] The concentration and form of the tritium is likely to be affected by the hydrological and geographical factors at the sampling location.[Momoshima et al 1991] During the period 1966-1968 the northern hemisphere inventory of tritiated methane was $4.3x10^4$ Bq kg $^{-1}$, a value fifty times weaker than that of molecular tritium during that period.[Belot et al 1996] Organically bound tritium on the other hand is only a recent consideration, and little is known about its evolution in the environment in the 1960's to 1980's.[Belot et al 1996]

Practically, tritium is often defined as 'loose' or 'bound', where loose tritium may be removed from plant or animal tissue by mild distillation techniques or lyophilization, and bound remains after this process and may be removed by combustion. [NCRP 1979] Bound tritium is then subdivided into two categories; stable or labile. The nature and thus the definition of OBT varies according to the system it is in. Exchangeable or labile OBT, is T which is bound to an electronegative atom such as oxygen, nitrogen or sulfur and is thus readily removable by exchange reactions with the hydrogen atoms of water or polar solvents. This form will be in equilibrium with the aqueous component of the system. Stable or non-exchangeable OBT, is T which is bound to a carbon atom and is only released during decomposition, or in the presence of strong acids, strong bases or catalysts.

2.2 Biological Effects and Risks associated with Tritium

The uptake of tritium by humans can occur by exposure to air, water or food. No external hazard is posed from it however, since the beta decay cannot penetrate the outer layer of dead skin cells, and hence tritium has a low radiological toxicity compared to other pure β emitters. It can enter the body by inhalation of atmospheric moisture, ingestion in drinking water or diffusion of vapour through the skin. Once inside the body, HTO diffuses freely and rapidly across cell membranes, equilibrating throughout the whole body water pool.[Momoshima et al 1991; Cawley and Lewis 1985] Chemical and biochemical processes also allow tritium to replace hydrogen in the body, resulting in a small fraction binding covalently to carbon in biomolecules, thus allowing retention in the form of organically bound tritium.[Osborne 1972; Johnson 1982] Taylor and Smith [1969] illustrated that carbohydrate metabolism is one of the major pathways in mammalian cells by which tritium from HTO can bind non-exchangeably to carbon.

OBT can enter the body by the ingestion of food as well as being produced in the body. Within the body, it tends to have an inhomogeneous distribution which also applies to individual organs and even within cells.[Momoshima et al 1991] As a consequence it tends to deliver doses to specific organs and cells rather than diluting itself throughout the 40 kg of water in the average human body. [Fairlie 1992] As a result, the accurate assessment of the contribution of OBT to the radiation dose is difficult to estimate. This is due to the uncertainties in quantitative data on the metabolic behaviour of ingested OBT, with particular regard to the quantities deposited and remobilised, and the residence time of different tissues. [Van Den Hoek et al 1985] Despite the uncertainties, the estimation of the accumulated dose in the human body due to tritium intake is an important subject for the protection of humans from radiation hazard caused by internally deposited tritium.[Saito 1998]

An intake of HTO becomes uniformly distributed throughout the body water within a few hours. [Pinson and Langham 1957; Osborne 1966] The residence time of T in the body is governed primarily by the turnover rate of body water and rises proportionally with body weight.[Diabate and Strack 1993] Half lives associated with the elimination of body water generally range from 8 to 12 days. [Hamby 1993] Trivedi et al [1997] carried out a series of experiments to assess the retention of tritium through urine analysis. Their results showed that the average biological half life of ^3H in body water is 6.3±1.0 days with increased fluid intake, and 8.4± 2.0 days with normal body fluid. The retention times of OBT in organisms are generally considerably longer than those of tritiated water and hence have a more significant consequence on dose estimates. Trivedi et al [1997] conclude from their studies that the dose from metabolised OBT contributes about 10% of the dose from tritium in the body water following acute HTO intakes.

The rate of metabolic degradation of non-exchangeable bound tritium varies depending on the type of organic molecule that contains the tritium atom, with clearance half times from minutes to hours for amino acids, and years for lipids and complex structural proteins such as collagen. OBT is present as so many different chemical compounds including amino acids, sugars, proteins, starches and cellulose (to name but a few) that it is not surprising that the variation in rates is so. [Smith 1969] The average biological half-life of T in metabolised OBT has been estimated by Trivedi et al [1997] as being 74± 18 days although, as stated before, this will depend on the tissue it is bound to. Animal studies carried out by Taylor [1989] suggest that with tritium-labelled foodstuffs containing amino acids or sugars, the proportion of T entering into C-H bonds is very variable. The relative tritium intake from HTO/OBT must be expected to vary according to the local situation, with the intake ratios of HTO/OBT depending on the national or regional situation, and to some extent, on the diet. [Taylor 1989] OBT is generally more radio-toxic than HTO because of its longer residence times in the body.

2.3 OBT in Biological Systems

OBT is taken into the human body and distributes itself inhomogenously amongst various organs and tissues depending on their chemical form. [Saito 1998] In biological systems OBT is evaluated by measuring the T distribution ratio (R-value), which relates to the tritium concentrations in organic matter to tissue water (TFWT). [Kim and Baumgartner 1991] The specific activity ratio of OBT:TFWT is assumed to be an indicator of the ability of the biological system to concentrate tritium into organic fractions. [Moghissi et al 1987] The possible exchange reactions between H of H_2O and labile organic H are the main source of error in the biological tritium determinations. [Mcfarlane 1976] At the time of formation OBT has essentially the same specific activity as the water from which it was formed, (OBT/HTO ratio = 1.0) since net isotopic fractionation in the biochemical reactions is slight. [Brown 1988] The concentration in lipids is always much higher than the concentration in proteins, and usually quite a lot higher than the concentration in total organic matter. The rate of activity loss in lipids is also slower. [Rudran 1988] The concentration of tritium per gram of organic matter in blood is always higher than that in urine and milk, and its retention is also longer.

2.4 OBT in Vegetation

The behaviour of tritium in plants, particularly crop plants, is an important consideration for the estimation of doses due to tritium release in the environment. In plants, tritium can be incorporated into organic molecules by metabolic processes dependent on light and reactions independent of light. Plants incorporate OBT over a much longer timescale than HTO and hence can provide not only a record of atmospheric tritium over the growing season, but also a relatively long-term history of the environmental tritium.[Watterson & Nicholson 1996; Hisamatsu et al 1998] The measurement of OBT in tree rings has been used to reveal historical environmental tritium levels. [Fuma and Inoue 1995]

Under the impact of light, organisms containing chlorophyl such as algae, mosses or higher plants, are able to synthesise organic compounds from inorganic carbon dioxide and water. As a consequence of the production of organic matter, tritium can be incorporated into such complex molecules as polysaccharides, proteins, lipids and nucleic acids. Photosynthesis in green plants, with HTO as a precursor, is the most important process in the photoinduced assimilation of non-exchangeable tritium. [Diabate and Strack 1993] The amount of OBT produced as a result however, is hard to predict quantitatively because the processes involved are influenced by a number of environmental factors, including; light, CO_2 and O_2 concentration, temperature, air circulation, H_2O supply, salinity, and diurnal and seasonal variations.[Diabate and Stack 1993; Bonotto et al 1984] In the absence of photosynthesis, the incorporation of OBT is low, suggesting that dark metabolism and/or exchange with hydrogen are limited.[Bonotto et al 1984] Experiments have been carried out to demonstrate the impact of light on the incorporation of OBT in plants. Moses and Calvin [1959] showed that in *chorella* algae there was a 3-fold increase in the incorporation of T into non-exchangeable positions during light than dark. Thompson and Nelson [1971] also demonstrated that there was a 10-fold increase in T assimilation in the light than dark. The small but not insignificant incorporation under night conditions, in leaves, stems and ears indicates therefore, that T can be incorporated into organic matter not only by photosynthesis but also by metabolic pathways independent of light.

The rate of tritium incorporation into edible plant parts, most of which are storage organs, depends on the development stage of the plant at the time of exposure. Indeka et al (1981) carried out experiments with the edible parts of tomatoes, potatoes, sunflowers, maize and wheat exposed to HTO in different stages of their growth. Tritium exposure in the generative period (after flowering) resulted in higher levels of incorporation into the organic matter than during the vegetative growth period. Algae on the other hand accumulate OBT as they grow, and once they have reached their stationary phase (the generative period), little tritium is converted into organic compounds. OBT increases rapidly in algae during the initial phase of growth, and once the stationary phase is reached, organic tritium is replaced with a half-life of about 150 days. It therefore appears unlikely that algae growing near an area of tritium release will transfer much organic tritium into the biosphere unless conditions for growth are very good. [Bonotto et al 1982] In a study with effluents released from a radiochemical laboratory, results have shown that tritium is preferentially incorporated into the organic matter of algae, but that the percentage of T which is biologically available is less than 1% of the total T present in the effluents. This fraction, although small, is however important for the contamination of the food chain.[Kirchmann et al 1979]

2.5 OBT in Soil

Tritium in soil water is derived from washout of tritiated vapour in air. [Fuma and Inoue 1995] When elemental atmospheric tritium comes into contact with soils it is rapidly converted to HTO by the activity of the micro-organisms. Only a small fraction of the T however, is directly incorporated from the HTO to OBT. The biological synthesis and consequently the uptake of tritium from HTO can be stimulated by the addition of energy sources such as glucose. [Diabate and Strack 1993; Forstel et al 1988]

The bulk of organic material in soils is permanently or periodically supplied by plants or plant residues. It is constantly degraded, altered, metabolised and remineralised by organisms. Organic material in upper soil layers typically consists of 70% of substances that are relatively easily decomposed, such as simple carbohydrates and cellulose. Other compounds such as lignin, resins and lipids are more resistant to microbial activity and contribute to the formation of humus. Where tritium is bound in non-exchangeable parts of the original plant material, this humic fraction may represent a long-term tritium reservoir in the soil, depending on the rate of decomposition processes. Decompositional processes convert the non-exchangeable bound tritium to HTO, which will be introduced into the soil water. [Diabate and Strack 1993]

Measurements of soil OBT originating from fallout were carried out by Bogen and Welford [1976] in the 1960s. Over a period of 7 years, from 1963-1970, they observed an exponential decrease in the tritium content of a soil from a sampling site in Oklahoma. From their findings they estimated a half-life of 3.5 years for OBT; corresponding to a mean residence time of 5 years.

2.6 Measurement of Tritium

A variety of methods are available for tritium measurement, and these vary according to the nature of the tritium, what form it is in and what it is bonded to. This project is concerned with environmental tritium measurements in sediments. Liquid scintillation counting is generally used to determine tritiated water activity, although more sensitive techniques such as ^{3}He mass spectrometry are also used.

Tritium in water samples is typically measured by recovering the tissue-free water from environmental samples using a series of methods; lyophilisation, distillation, azeotropic distillation and dilution being the most prevalent. Each of theses techniques has its merits and disadvantages.

Lyophilisation involves freezing the sample and recovering the water under vacuum. The water is generally recovered in a cold finger located between the vacuum and the sample. The disadvantage of this technique is the time required to collect enough water for low-level environmental sampling, which can be up to several days. [Wood et al 1993] Distillation involves heating the sample and collecting the distillate on a cold finger. Depending on the accuracy of measurements required, isotopic fractionation should be taken into consideration. The possibility of isotopic fractionation can be eliminated if the sample is completely distilled. Azeotropic distillation involves placing the sample in an immiscible hydrocarbon, eg toluene or xylene and the water (in the sample) is boiled out as a constant boiling mixture of fixed water-hydrocrabon composition. When taken to completion, there is no isotopic fractionation. The disadvantage of this technique is that the dried organic residue must be free of hydrocarbon if any organically bound tritium measurement is desired on the same samples. [Wood et al 1993] Dilution of a sample is a simple technique to recover HTO. If left long enough, the water in the sample will reach equilibrium with the water added. Adding heat can accelerate the process. The disadvantage of this method is that the sample is diluted by the solvent, and therefore it is usually only used when the tritium concentrations are significantly higher than the background levels. [Wood et al 1993]

OBT is typically determined by the separation of the water component (tissue-free water) by one of the methods described above, from other forms of tritium. The difference between the total tritium measurement and the tritiated water fraction results in OBT. With solid samples, tritiated water is generally removed by drying or lyophilisation, and the dried material combusted in an oxygen enriched atmosphere or chemically oxidized to convert the different forms to water for analysis. A method for OBT measurements described by Moghissi et al [1975] involves placing a sample in a 2-L steel Parr bomb, pressurised with oxygen to about 1.5-2 MPa and ignited. The bomb is then evacuated through a cold trap to collect the water. The advantage of this method is that it is very quick and the water does not require further purification, however only about 10g of organic material can be combusted at once. Larger samples are generally combusted in a quartz tube with controlled oxygen and gas flows. The water fraction recovered from heating the sample usually requires purification; this is achieved by refluxing with $KMnO_4$ and/or Na_2O_2. [Hisamatsu et al 1990b] The pure sample is then counted using liquid scintillation counting. There is reasonably good agreement found across the various techniques as Hisamatsu et al [1990b] illustrated in an intercomparison exercise with six participating laboratories.

Liquid scintillation counting is the most common procedure used for measuring tritium in water. The water samples to be analysed are mixed with a liquid scintillation 'cocktail' in a polyethylene scintillation vial. The vials generally have a capacity of 20-24ml, and typical water:cocktail ratios used are between 1:5 and 1:15 for routine applications. [Wood et al 1993] In this method the sample is placed between 2 photomultiplier tubes. The beta radiation in the sample interacts with the scintillant, resulting in several light photons being emitted. The summed height of the pulses from the two photomultiplier tubes is proportional to the energy of the beta particle. The counters are also run in coincidence in order to distinguish photon pulses from photomultiplier noise. [Wood et al 1993]

The ^3He mass spectrometry technique provides ultimate sensitivity for tritium measurement. The tritium sample in water is purged of ^3He, stored and sealed in a container for several months. The sample is then analysed for ^{3}He growth resulting from tritium decay, using a mass spectrometer. Although several months of storage time is required for this technique, mass spectrometer measurements only require 30 minutes.

2.7. References

Belot, Y., Roy, M., Metivier, H., Le Tritium de l' Environment a l'Homme. Institut de Protection et de Surete Nucleare, Les editions de Physique, 1996.

Bogen, D.C., Welford, G.A., *Fallout tritium distribution in the environment,* Hlth. Phys., **30**, p. 203-208, 1976.

Bonotto, S., Arapis, G., Mellet, J., Hoursingon-Neubrium, D., Cinelli, F., Belcari, P., Gerber, G.B., Kirchmann, R., *Behaviour of tritium in the marine environment: Uptake and distribution of tritiated water and of tritiated organic compounds in unicellular and multicellular algae*, Hydrobiologia, **116/117**, p. 187-200, 1984.

Bonotto, S., Gerber, G.B., Arapis, G., Kirchmann, R., *Modelization of tritium transfer into the organic compartments of algae*, Ann. Ass. Belge., Radioprotection, **7**, p. 283-292, 1982.

Brown, R.M., *Assessment of the significance of organically-bound tritium in environmental materials*, A.E.C.B., Ottawa, ON(Canada). Sep. 1988, p.35

Buttlar, H., Libby, W.F., *Natural distribution of cosmic-ray produced tritium*, J. Inorg. Nucl. Chem., **1**, 75, 1955.

Cawley, C.N., Lewis, B.A., *Tritium technology and labeling*, I. Trans. Am. Nucl. Soc., **50**, p. 39, 1985.

Craig, H., Lat, D., Tellus, **13**, p. 85, 1961.

Diabate, S., and Strack, S., *Organically bound tritium*, Hlth. Phys., **65**, p. 698-712, 1993.

Diabate, S., Stack, S., *Organically bound tritium in wheat after short-term exposure to atmospheric tritium under laboratory conditions*, J. Environ. Radioact., **36**, p. 157-175, 1997.

Etnier, E.L., Travis, C.C., Hetrick, D.M., *Metabolism of organically bound tritium in man*, Rad. Res., **100**, p. 487-502, 1984.

Fairlie, I., The Ecologist, **22**, 5, p. 228-232, Sep/Oct 1992

Forstel, H., Papke, H., Hilmann, I., *Uptake of tritium in the organically bound form into the biomass of the soil*, Fus. Tech., **14**, p. 1258-1263, 1988.

Fuma, S., Inoue, Y., *Simplified and sensitive analysis of organically bound tritium in tree rings to retrospect environmental tritium levels*, Appl. Radiat. Isot., **46**, p. 991-997, 1995.

Hamby, D.M., *A probabilistic estimation of atmospheric tritium dose*, Hlth. Phys., **65,** p. 33-40, 1993.

Higgins, N.A., *TRIF-An Intermediate Approach to Environmental Tritium Modelling*, J. Environ. Radioact., **36**, p.253-267, 1997.

Hill, R.L., and Johnson, J.R., *Metabolism and dosimetry of tritium*, Hlth. Phys., **65**, p.628-647, 1993.

Hisamatsu, S., Katsumata, T., Takizawa, Y., Inoue, Y., Isogai, K., Kim, J., Katagiri, H., Takashima, Y., Kaji, T., Nagatani, F., Ueno, K., Itoh, M., *Interlaboratory comparison of low-level organic tritium measurement in environmental samples*. Radioisotopes, **39**, p.457-463, 1990b.

Hisamatsu, S., Amano, H., Isogai, K., Atarashi, M., Zhu, H., Takizawa, Y., *Organically-bound 3H concentration in rice around atomic energy facilities*, Hlth. Phys., **74**, p. 448-450, 1998.

Indeka, L., *Incorporation of tritiated water from the atmosphere into aqueous and organic components of plants.* In: Jaworowski, Z., ed. Biological incorporation of tritium. Warsaw, Poland: Central Laboratory for Radiological Protection; CLOR-115/D; p. 21-27, 1981.

Johnson, J.R., *The estimation of the effective dose equivalent from tritiated water exposures using tritium concentrations in urine.* Radiat. Prot. Dosim. **2**, p.245-247, 1982.

Kaufman, S., Libby, W.F., *The natural distribution of tritium*, Phys. Rev., **93**, p.1337, 1954.

Kim, M.A., and Baumgartner, F., *Tritium fractionation in biological systems and in analytical procedures*, Radiochimica Acta, **54**, p. 121-128, 1991.

Kirchmann, R., Bonotto, S., Soman, S.D., Krishnamoorthy, T.M., Jyengar, T.S., Moghissi, A.A., *Transfer and incorporation of tritium in aquatic organisms,* Part of I.A.E.A report on the Behaviour of tritium in the environment, p. 187-204, 1979.

Mcfarlane, J.C., Environm. Exp. Bot., **16**, p. 9, 1976.

Moghissi, A., Brethauer, E.W., Platt, W.F., McNelis, D. N., *Oxygen bomb combustion of environmental and biological samples for tritium analysis,* Int. J. Appl. Radiat. Isotopes, **26**, p.339-342, 1975.

Moghissi, A., Brethauer, E.W., Partzer, R.G., *Biological concentration of 3H*, Hlth. Phys., **53**, p. 385-388, 1987.

Momoshima, N., Okai, T., Kaji, T., Takashima, Y., *Distribution and transformation of various chemical forms of tritium in the environment.* Radiochimica Acta, **54**, p. 129-132, 1991.

Moses, V., Calvin, M., *Photosynthesis studies with tritiated water,* Biochim. et Biophys. Acta, **33**, p. 297-312, 1959.

National Council on Radiation Protection and Measurement. A handbook of radioactivity measurements procedures.2nd ed. Bethesda, MD:NCRP; Report 1985:368.

NCRP, Tritium in the environment, Report no. 62, National Council on Radiation Protection and Measurements, Washington DC., 1979.

Okada, S., Momoshima, N., *Overview of tritium: characteristics, sources and problems.* Hlth. Phys., **65**, p. 595-609, 1993.

Okai, T., Takashima, Y., *Tritium concentration in atmospheric water vapor, hydrogen and hydrocarbons in Fukuoka.* Appl. Radiat. Isotopes. **42**, p.389-407, 1989

Osborne, R.V., *Absorption of tritiated water vapour by people.* Health Phys., **12**, p. 1257-1537, 1966.

Osborne, R.V., *Permissible levels of tritium in man and the environment.* Radiat. Res., **50**, p. 197-211, 1972.

Ostlund, H.G., *Sampling system for atmospheric tritium gas (HT) and tritiated water (HTO).* IEEE Trans. Nucl. Sci. **21**, p.510-512, 1974.

Pinson, E.A., Langham, W.H., *Physiology and toxicology of tritium in man*. J. Applied Physiol., **10**, p.108-126, 1957.

Rozanski, K., Gonfiantini, R., Araguas-Araguas, L., *Tritium in the global atmosphere: Distribution patterns in recent trends*. J. Phys. Part G: Nucl. Part. Phys. **17**, p. 523-536, 1991a.

Rudran, K., *Significance of In Vivo organic binding of tritium following intake of tritiated water*, Rad. Protect. Dosim., **25**, p. 5-13, 1988.
Saito, M., *Blood tritium level as a conservative estimate of soft tissue dose after tritium injection in mice*, Hlth. Phys., **74**, p. 561-567, 1998.

Smith, T.E., Taylor, R.T., *Incorporation of tritium from tritiated water into carbohydrates, lipids and nucleic acids*, Lawrence Livermore Radiation Laboratory, Livermore, U.S Report UCRL-50781; 1969.

Taylor, D.M., *An approach to the proposal of an annual limit of intake for the general public (ALIgp) for organically bound tritium*, Rad. Protect. Dosim., **28**, p. 211-213, 1989.

Thompson, R.G., Nelson, C.D., *Photosynthesic assimilation and translocation of* ^{3}H*- and* ^{14}C*-organic compounds after* ^{9}HHO *and* $^{14}CO_2$ *were simultaneously offered to a primary leaf of soybean*. Can. J. Bot., **49**, p.757-766, 1971.

Trivedi, A., Galeriu, D., Richardson, R.B., *Dose contribution from metabolised organically bound tritium after acute tritiated water intakes by humans*, Hlth. Phys., **73**, p. 579-586, 1997.

United Nations: Ionizing radiation: Sources and biological effects. United Nations

Scientific Committee on the Effects of Atomic Radiation. Report to the General Assembly, with Annexes, New York 1982.

United Nations: Sources and effects of ionizing radiation. United Nations Scientific Committee on the Effects of Atomic Radiation. Report to the General Assembly, with Annexes, New York 1977.
United Nations: Sources, effects and risks of ionizing radiation. United Nations Scientific Committee on the Effects of Atomic Radiation. Report to the General Assembly, with Annexes, New York 1988.

Van Den Hoek, J., Ten Have, M.H.J., Gerber, G.B., Kirchmann, R., *The transfer of Tritium-Labeled Organic Material form Grass into Cow's Milk*, Radiat. Res. **103**, p.105-113, 1985.

Van Soy, K.A., Fine, A.F., Ostlund, H.G., *Two decades of mixing tritium into the North Pacific Ocean.* Deep Sea Res. **38**, p.191-219, 1991.

Warwick, P.E., Croudace, I.W., Howard, A.G., *Improved technique for the routine determination of tritiated water in aqueous samples*, Analytica Chimica Acta, **382**, p. 225-231, 1999.

Watterson, J., Nicholson, K.W., *Change in radionuclide content of crops as a result of food preparation*, J. Radiol. Prot., **16**, p. 191-200, 1996.

Wood, M.J., McElroy, R.G.C., Surette, R.A., Brown, R.M., *Tritium sampling and measurement*, Hlth. Phys., **65**, p.610-627, 1993.

3. Tritium Measurements

3.1 Introduction

One of the objectives of this study is to establish a working definition for Organically-Bound tritium, non-aqueous tritium and tritiated water in the context of environmental processes, and to develop analytical protocols to distinguish between these various forms of tritium.

In 1999 the Environment Agency commissioned an inter-laboratory comparison study in which 7 laboratories analysed effluent from Nycomed-Amersham for tritiated water and OBT using their own methods. This study concluded that the most acceptable and apparently reliable methods involved wet oxidation of a sample or combustion in a high temperature furnace and that the most practicable approach to analysing OBT is to assume that it is the total tritium content of a sample once the free tritiated water component has been excluded. [Ware & Allot 1999] This chapter aims to outline the procedures involved in the development of the analytical protocol employed for the tritium measurements in this study based on information from this exercise.

3.2 ^{3}H Analysis

Practically, tritium is defined as 'loose' or 'bound' where loose tritium may be removed from plant or animal tissue by mild distillation techniques or lyophilization, and bound remains after this process and may be removed by combustion. [NCRP 1979] Bound tritium (OBT) is then subdivided into stable and exchangeable T, where exchangeable tritium is readily removable by exchange reactions with the hydrogen atoms of water or polar solvents, and will be in equilibrium with the aqueous component of the system. Stable OBT, is bound to a carbon atom and is only released during decomposition, or in the presence of strong acids, bases or catalysts. To determine the nature of the tritium found in samples, two techniques were employed to measure both water extractable tritium and total tritium. The difference between these two measurements would result in the amount of OBT found in a sample.

3.3 Liquid Scintillation Counting

3.3.1 Introduction

Liquid scintillation counting is commonly used for the measurement of beta radioisotopes such as tritium. All the tritium measurements in this study were done on a Wallac 'Quantulus' scintillation counter.

3.3.2 Wallac 'Quantulus ' scintillation counter

Like all scintillant counters, the Wallac 'Quantulus' consists of photomultiplier tubes that detect photons emitted from a sample vial, during the scintillation process. The number of counts generated over a time period as well as their intensity is measured and recorded by the counter. The intensity of the photons is directly related to the energy of the beta particles that initiate the process. A multichannel analyser (MCA) is incorporated which analyses separately the number of counts and the intensity and an energy spectrum is derived which allows the identification of the radioisotopes present in the sample.

A Wallac scintillation counter was employed for this study because unlike conventional scintillation counters it has an additional 2 photomultiplier tubes set in anti-coincidence with the other photomultiplier tubes. This enables the detection and seperation of 'true' events and cosmic background events. When an event is measured in both sets of photomultipliers it is regarded as a cosmic background event and is disregarded. The Wallac 'Quantulus'counter can thus differentiate between what is a genuine decay event from a sample and what is as a result of cosmic background event.

3.3.3 Scintillant cocktail composition

Liquid scintillation counting involves using a scintillant mixture or cocktail that enables the energy associated with radioactive decay, to be converted into light photons that can then be detected by the photomultiplier tubes. The converted light photons produced can however also interact with other compounds or molecules found in the cocktail, before reaching the photomultiplier tubes. This process results in lower numbers of events registered as the photons may no longer be of the correct energy to be detected, they may simply be absorbed by another molecule or they may physically be unable to reach the detector due to obstruction of matter. This reduction in counting efficiency is known as quench, which is sub divided into chemical, colour and physical quench. The composition of the scintillant therefore affects the number of solvent molecules excited and thus the counting efficiency.

3.3.4 Scintillant cocktail efficiency experiment

In order to determine the most suitable scintillant cocktail to use for this study 6 scintillants were tested for the optimum counting efficiency and water : scintillant composition. These scintillants were chosen as they are commonly used for tritium analysis and some of them are specifically for 'low level tritium' (LLT) determination.

The 6 scintillants chosen were:

1. Ultima Gold^{tm} AB (LLT) (Packard)
2. Gold Star (Meridian)
3. Picofluor LLT (Packard)
4. Optiphase 'Trisafe' (Wallac)
5. Aquasafe 500 plus (Zinser Analytic)
6. Aquasafe 300 plus (Zinser Analytic)

A series of blanks and standards were prepared in 20ml polyethylene vials using the 6 scintillants and de-ionised water. The standards consisted of 7,8,9,10,12 ml of water and made up to 20ml with scintillant accordingly. A 200µl aliquot of T-standard (90.12Bq/g) was spiked into each of the vials. The blank samples consisted of the same volumes of water: scintillant without the addition of the tritiated standard. The sample vials were left in the Wallac 'Quantulus' counter overnight to minimise the effects of chemiluminescence and photoluminesecence.

3.3.5 Results

The efficiency for each of the scintillants was calculated and plotted against water content. With increasing water content the scintillant counting efficiency decreases, as is illustrated in Figure 3.1

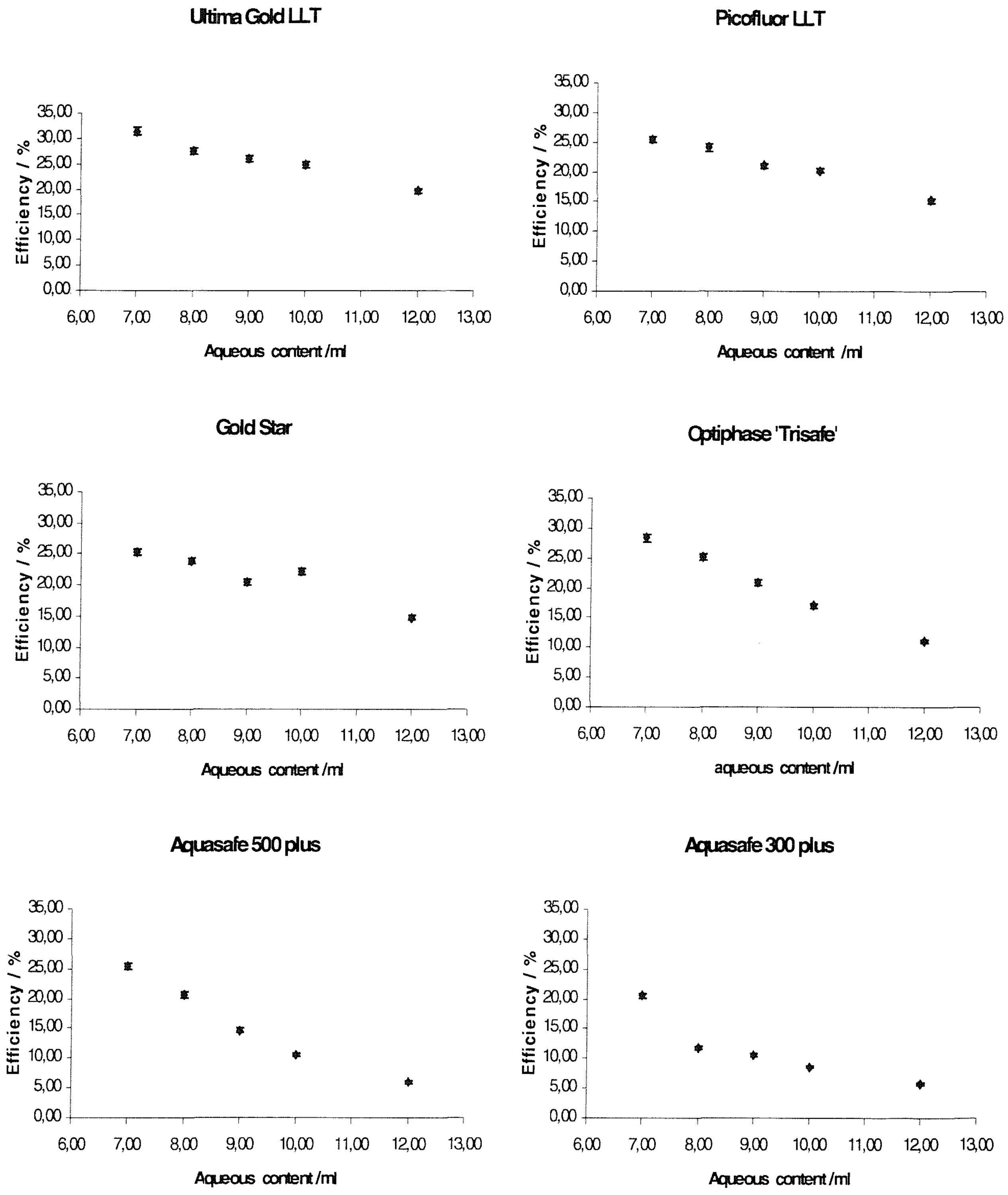


Figure 3.1: Counting efficiency of scintillation cocktails in water.

The efficiency of the scintillants decreased with increasing water content. Ultima Gold, Picofuor and Gold Star showed similar efficiencies. Gold Star was however chosen as the scintillant of choice instead of Ultima Gold LLT and Picofluor LLT as it was a more cost effective and showed sufficient efficiency to be used for the purposes of this study. The water volume used throughout this study was 8ml which had a counting efficiency of 23.88% used for all calculations.

The counting efficiency for seawater and toluene were calculated in the same fashion, a series of samples were spiked with a known activity of tritium and counted repetitively so that an average efficiency was obtained. The raw efficiency values used throughout this study are illustrated in Appendix 2.0 along with the calculations used to derive them.

3.3.6 Background Reproducibility

Tritium is a difficult radionuclide to measure because it is naturally present in water with 3 exceptions;

1. Bore hole water, water older than 120 years. Any available tritium would have decayed because the half-life of tritium is 12.35 years and the water would have not been exposed to nuclear weapons fallout.
2. Ice from Icebergs frozen and maintained below water.
3. Water trapped during mineralisation, calcium sulfate often used (water of crystallisation).

Even when these waters types are used in the liquid scintillation counter, a background reading is seen. This background may be due to:

- If vials employed are made from glass, higher backgrounds are seen due to the presence of ^{40}K.
- Electronic noise present in the machine is the main cause and can never really be gotten rid of.
- Cosmogenic interferences can also cause increased background.

In order to come to an average value for distilled water (RO water), the main type of water employed during this study, 5 blank samples were made up, each consisting of 8ml of water and 12ml of Goldstar scintillant, and counted in succession 4 times for 60 minutes. Two of the samples were then counted repeatedly for 120 minutes. The raw results are shown in Appendix 2.1 The table illustrates the higher level of delayed coincidence for samples counted for a shorter amount of time, as less time is available for background levels to drop. Samples counted throughout this study were counted for 120 minutes to lower the amount of delayed coincidence measurements. A background value of 1.68Bq was assumed for all the calculations.

3.4 Water Extractable Tritium

Water-extractable tritium was estimated by mixing an accurately weighed subsample of ~ 5g of fresh sediment (the wet sediment used was first stirred to make it homogenous) with 10ml of deionised water. The sample was allowed to equilibrate for 1 hour on a mixing wheel, centrifuged for 20 minutes, and then filtered through a 0.45 μm Cyclopore track etched membrane filter. The filtrate was then made up to 25ml and an 8ml aliquot was used for the tritium determination.

3.5. Total Tritium

3.5.1 Overview

Total tritium measurements were obtained by combusting a sample of known mass, in a combustion furnace in an oxygen enriched atmosphere. The sample combustion converted tritiated species to tritiated water which was collected and measured using liquid scintillation counting.

3.5.2 Furnace Design

3.5.2.1 Initial set-up

A Lenton (ref.15032) special horizontal tube, 2 zone furnace was used to release total tritium from sediment samples. The Zone 1 of the furnace was a "cold" temperature zone, with temperatures ranging from room temperature to 500 °C. The high temperature zone (Zone 2) of the furnace could be set to temperatures up to 1600°C. These zones are illustrated in the temperature profile in Figure 3.2 and photographically in Figure 3.3. The quartz combustion tube, held ~ 10g of a 0.5% Pt/Alumina (Johnson Matthey, 0.5% platinum on 3mm alumina pellets) within Zone 2. This catalyst was initially chosen because it had proved to be useful in another laboratory (Harwell) carrying out similar work.

The temperature distribution within the furnace was assessed using a thermocouple. The warmest part of Zone 1 was situated near the middle of the zone (~30 cm along), and was chosen as the area in which the sample would be placed. Figure 3.2 illustrates the temperature profile of the furnace set-up.

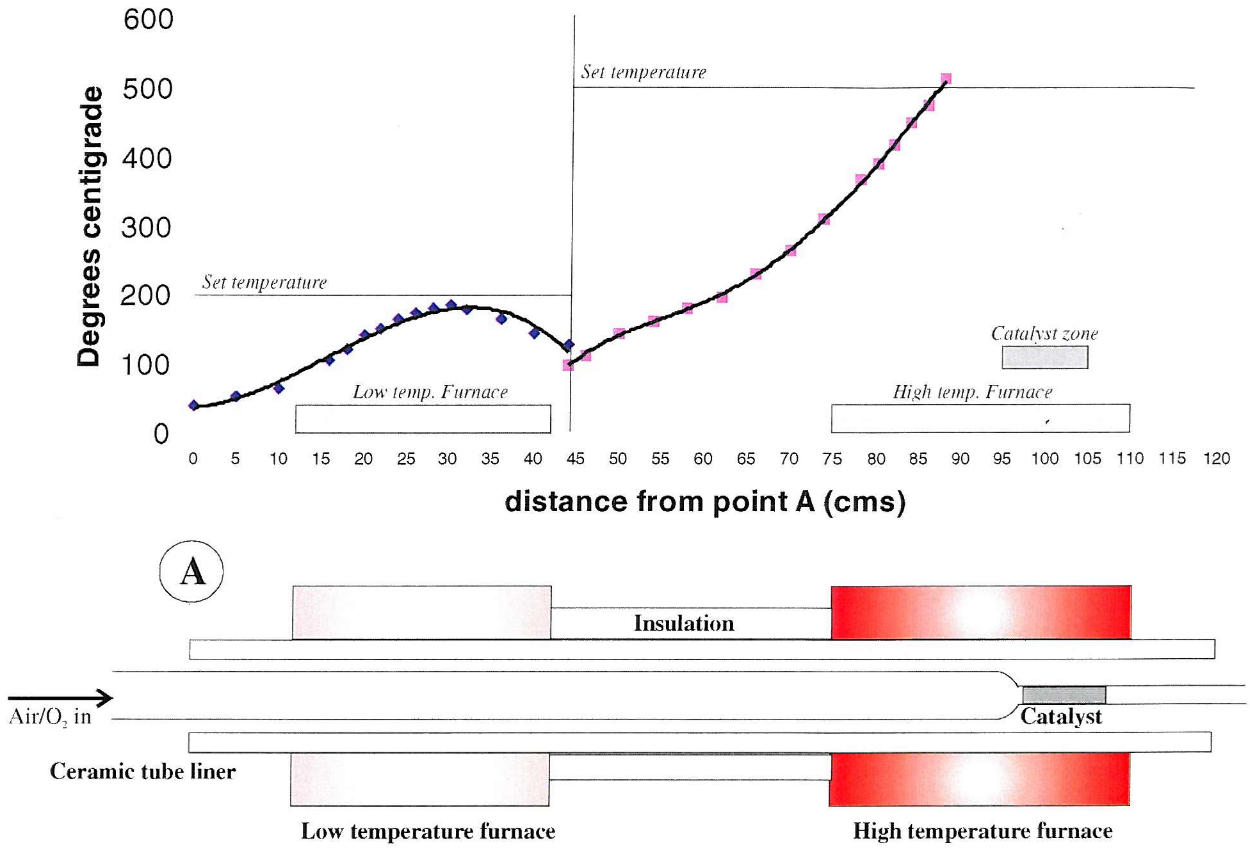


Figure 3.2: Temperature profile of furnace set-up.

The set-up designed for total tritium measurements is shown in the photographs in Figure 3.3. The furnace was fitted with 4 quartz combustion tubes in order to maximise sample throughput. Each tube contained the same amount of catalyst and was subjected to the same conditions. Gas flows into the system were monitored using flow-meters. The gas flow and its composition depended on the required; air/oxygen ratio, the bubbler size and the furnace tube volume. Gas flows that were too slow could lead to incomplete combustion of the sample in a given time period. Too great a gas flow would lead to excessive loss of water from the bubblers, due to vigorous bubbling and increased evaporation as the gas leaving the tube was in the form of steam. A gas mixture of 150ml/min oxygen and 220ml/min air was chosen for the initial runs.

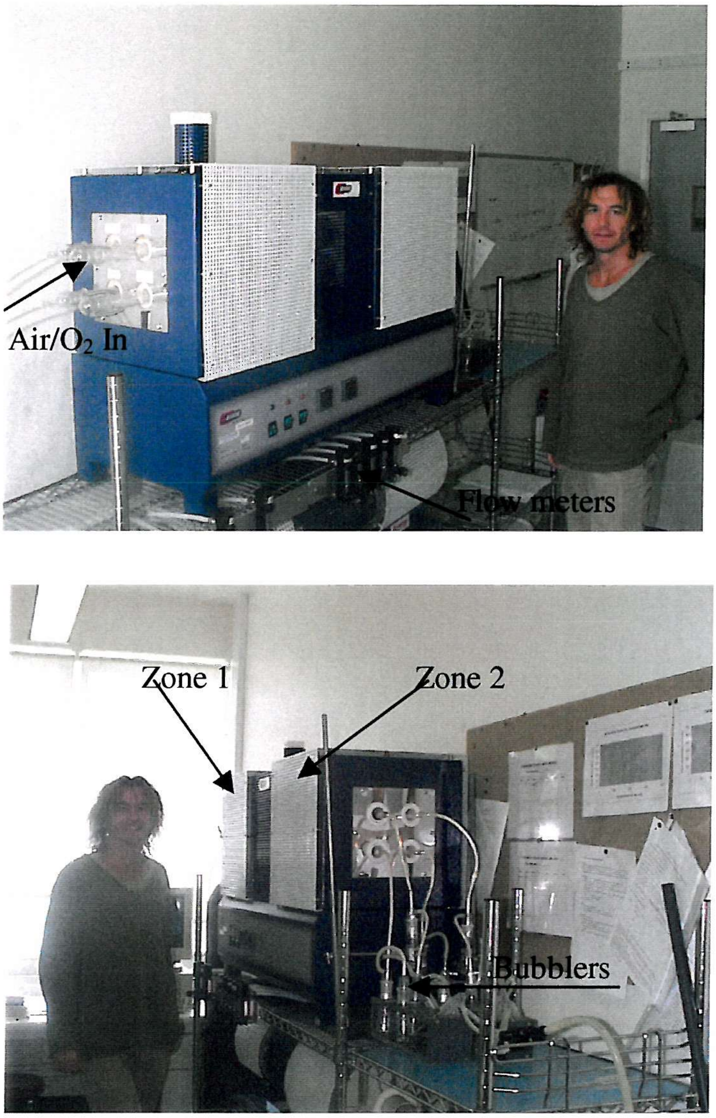


Figure 3.3: Photograph illustrating the furnace set up.

The furnace set up is shown in Figure 3.3. The glassware was specially designed to fit into the furnace, and the dimensions of a quartz tube used for total tritium measurements is shown in Figure 3.4 along with a bubbler and a socket used. The air/oxygen gas mixture flows along the tube and over the sample (placed at the coloured section of the tube). The volatilised and partially combusted products then pass over the catalyst, through the PTFE tubing to a bubbler filled with 20ml of distilled water.

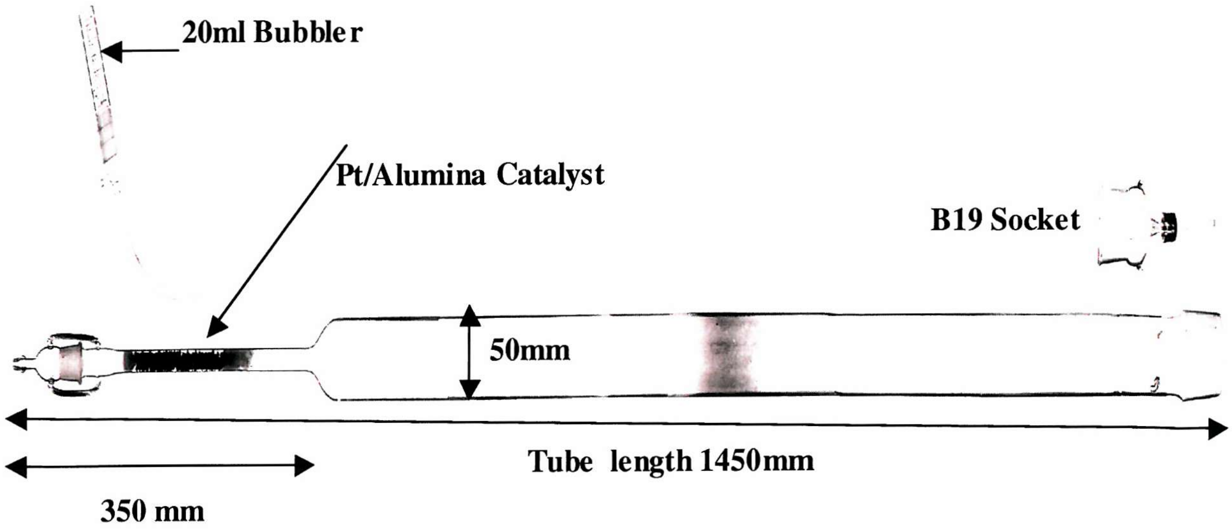


Figure 3.4: Glassware picture and dimensions

3.5.2.2 Furnace Optimization

Initial evaluation of the furnace was carried out by combusting a 100µl aliquot of a ~0.75KBq/g tritiated-thymidine standard, with zone 1 set at 25°C and ramped to 200°C with a ramp rate of 5°C/min, and zone 2 set at 500°C. A gas mixture made up of 150 ml/min O_2 and 220 ml/min air was passed through the combustion tube for 4 hours, changing the bubblers every 30 minutes. The constant changing of the bubblers was used to monitor tritium recovery with change in temperature. Initially recoveries were very poor and very inconsistent. It was decided that the poor recoveries might have been due to inefficient combustion of the sample by the catalyst, and therefore the temperature of zone 2 was increased to 800°C. Recoveries increased and were consistently high, as shown in Table 3.1.

Date	Pt/Al Catalyst Temp. °C	Recovery / %
05/06/00	500	25.3± 2.4
07/06/00	500	77.3± 2.4
12/06/00	500	77.3± 5.2
14/06/00	500	82.2± 4.6
16/06/00	500	20.1± 1.4
20/06/00	500	87.2± 2.7
23/06/00	500	35.0± 2.2
19/09/00	800	97.5± 2.7
16/05/01	800	89.8± 2.6
16/05/01	800	86.9± 2.6
16/05/01	800	97.7± 2.8

Table 3.1: Recovery of tritium from a tritiated-thymidine standard with changing catalyst temperature

The recovery of tritium from a tritiated-sugar standard, composition sucrose: dextran: D-galactose 1:1:1 (by weight), was measured over time as temperature of zone 1 increased. The sample was placed in Zone 1, already at 140°C and the zone was ramped up to 500°C using a ramp rate of 5°C/min. The recovery of tritium was 82.6% in the first 90 minutes (furnace by then at 290°C) and at 150 minutes (500°C) 90% has been recovered (Figure 3.5). The run times were therefore changed to 150 minutes, as there was little increase in recovery at this time. Recoveries also benefited from less water being lost from the bubblers, through evaporation.

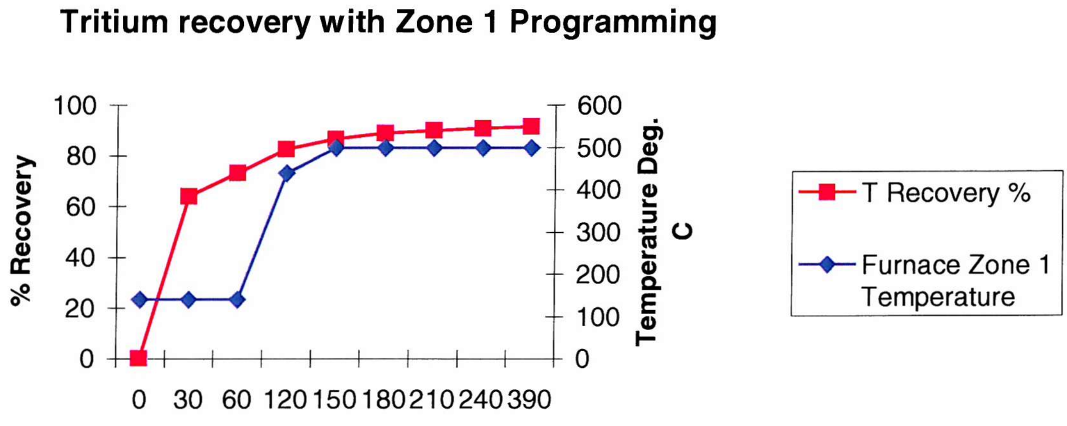


Figure 3.5: Recovery of tritium from a ^{3}H-sugar standard during the temperature programming of Zone 1 furnace

Ultimately, furnace Zone 1 was set at 90°C and then ramped to 500 °C , with a ramp rate of 5°C/min, the temperature of Zone 2 was fixed at 800°C. The constant changing of the bubblers was abandoned once optimum conditions had been established.

3.5.3 Final Procedure

Total tritium was determined by combusting ~10g of fresh, wet sediment in a purpose-designed furnace using a predetermined heating cycle up to 500°C, in an oxygen enriched atmosphere. The combustion products were passed over a Pt-Alumina catalyst, heated to 800°C, in order to ensure the complete combustion of tritiated species to tritiated water. The tritiated water was trapped in 2 bubblers, connected in series, filled with 20ml of deionised water. An 8ml aliquot from each bubbler was taken to determine the tritium concentration by liquid scintillation counting. Blank and tritiated sucrose standards were run every 5 samples to monitor the performance of the furnace, and to detect furnace background values and catalyst failure.

3.5.4 ^{3}H-sucrose Recoveries

In order to assess the performance of the combustion furnace, sediment samples were spiked with a tritiated sucrose standard and analysed repeatedly. The sucrose standard used was supplied by Nycomed- Amersham and had an activity of 37 KBq/ml. A 50µl aliquot was spiked onto ~5g of dried, tritium free sediment in an alumina crucible. The crucible was then placed in the furnace, and heated using the predetermined protocol. This same ^{3}H-sucrose spiked sediment sample was used as a control standard in subsequent studies and run every 5 samples to monitor the performance of the furnace. Recoveries were high, averaging 95.6 ± 9.97 %. Recoveries were also monitored to establish a working lifetime for the catalyst. The catalyst was deemed to have failed if recoveries dropped below 80%. On average the Pt-Alumina catalyst was effective for ca. 25 sediment combustions. The recovery chart is shown in Figure 3.6.

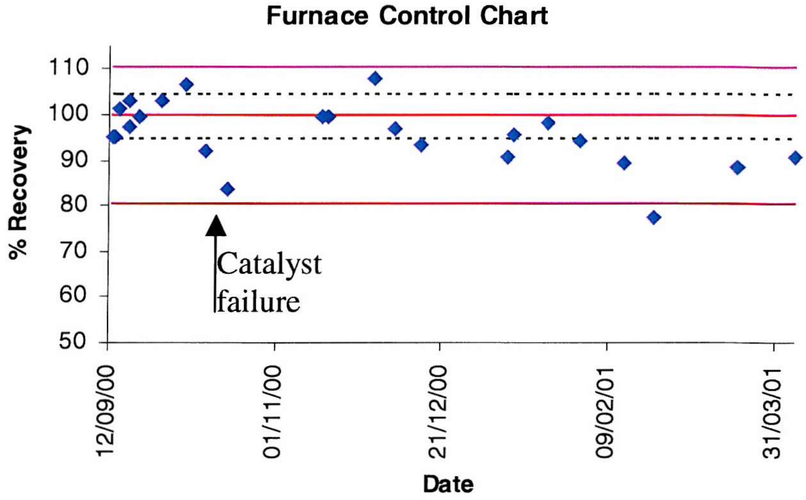


Figure 3.6: ^{3}H sucrose recoveries

3.5.5 Recoveries of ^{3}H from other labelled compounds

The routine monitoring of the furnace performance was achieved by using the ^{3}H-sucrose standard. This standard is however, not necessarily representative of the type of tritiated organics present in the Cardiff bay area. It may not accurately reflect the recoveries of other, potentially more thermally stable compounds that may be present, in either the sediments themselves or in the discharges from Nycomed- Amersham. A series of standards was therefore chosen to better reflect the range of compounds present in the discharge. The recoveries were monitored using 2 commonly used catalysts, Pt/Alumina and CuO/Alumina. The Pt/Alumina catalyst was supplied by Johnson Matthey, (0.5% platinum on 3mm alumina pellets), and the CuO/Alumina catalyst was Aldrich (copper (II) oxide, 13%wt on alumina). All the recoveries were high, exceeding 90%, although recoveries using the Pt/alumina catalyst were generally higher. The results are shown in Table 3.2.

Compound Class	Composition (Ratio by wt)	Recovery Pt-alumina	Recovery Cuo-alumina
Nucleotides	thymidine 5'-triphosphate: deoxyadenosine 5'-triphospahe 1:1	95 ± 06.%	82 ± 0.2%
Hydrocarbons	DMBA (dimethylbenzanthracene) : benzopyrene 1:1	93 ± 0.7%	79 ± 0.2%
Thymidine		98 ± 2.7%	99 ± 2.9%
Amino Acids	leucine: lysine: phenylalanine: proline: tyrosine, 1:1:1:1:1	97 ± 0.6%	87 ± 0.2%
Methanol Waste	tritiated water: methanol, 85:15	98 ± 0.6%	87 ± 0.2%
Sugars	sucrose: dextran: D-galactose, 1:1:2	95 .6 ± 10%	90 ± 0.7%
HTO	Water	100%	n/a

Table 3.2: Furnace recoveries using a series of ^{3}H-labelled compounds

3.5.6 Furnace Blanks

In order to ensure that the measurements obtained from the combustion of Cardiff bay samples were genuine, a series of blank samples was run. A blank result, as defined by Currie [1968], is 'the signal resulting from a sample which is identical, in principle, to the sample of interest, except that the substance sought is absent (or small compared to the standard deviation).The blank thus includes the effects of interfering species.' Blank sediment samples were chosen because of their ease of collection and availability. The Cumbrian samples were collected from sites near to Sellafield, and were used as control samples. The blank sample results are shown in Table 3.3.

Blank samples run in the furnace also provided a limit of detection for sample analysis. A detection limit is defined as a 'minimum detectable true activity' by Altshuler and Pasternack [1963]. The significance of this definition is that it allows the determination, for a given measurement process, the smallest (true) signal which will be detected. The limit of detection ranged from 0.01-0.03 Bq/g with a 95% confidence level. This was calculated using 'working expressions for radioactivity' derived by Currie [1968] for paired observations.

Date	*Site*	*Acitvity Bq/g*
12/03/01	Itchen, Southampton	<0.03
12/03/01	Itchen, Southampton	<0.02
20/10/00	Maryport, Cumbria	<0.01
20/10/00	Workington, Cumbria	<0.09
20/10/00	Whitehaven, Cumbria	<0.011
20/10/00	Ravenglass, Cumbria	<0.015

Table 3.3; Activities of blank sediment samples

3.5.7 Measurement reproducibility

A sediment sample from Peterstone-Great Wharf (sampling site 5), taken on 17/05/00, was sub-sampled and the 4 sub-samples were analysed for total tritium using the combustion method (Section 3.5.3). The results are illustrated in Table 3.4.

Activity Bq/g	*Error (2SD)*
0.725	0.020
0.755	0.021
0.724	0.025
0.751	0.028

Table 3.4; Sample reproducibility

3.5.7.1 Conclusions

The results showed good agreement and were within 2 standard deviations of each other. This indicated that not only was there good reproducibility with the apparatus, but also that the samples were homogenous.

3.5.8 The affect of sample mass on tritium measurements

The affect of sample mass on response had to be investigated in order to ensure that recoveries were not dependent on the size of the sample taken for analysis. The sample used had previously been used to check for sample reproducibility (Section 3.5.7), and hence the confidence in the sample value was high.

3.5.8.1 Method

Sub-samples of the Peterstone 17/05/00 sample, having masses ranging from ~0.5 to 10.0g were taken. The exact mass was noted and the samples were combusted in the furnace, following the protocol described in Section 3.5.3. An 8ml aliquot was then used for liquid scintillation counting.

3.5.8.2 Results and Discussion

The tritium recovered from samples of varying mass is shown in Figure 3.7. The maximum and minimum lines represent the maximum and minimum values obtained from the reproducibility exercise (Section 3.5.7), with a minimum value of 0.70Bq/g and a maximum of 0.78Bq/g.The sample error decreases with increasing mass, and the variation in activity decreases once the sample mass is greater than ~ 3g. The optimum mass for sample measurements therefore is between 3 and10g, 5g therefore appears to be a reasonable sample mass. The responses from samples of differing mass however appear to be within error of each other, with overlap around 0.75 Bq/g. This observed variation is typical of sampling errors associated with sample inhomogeneity.

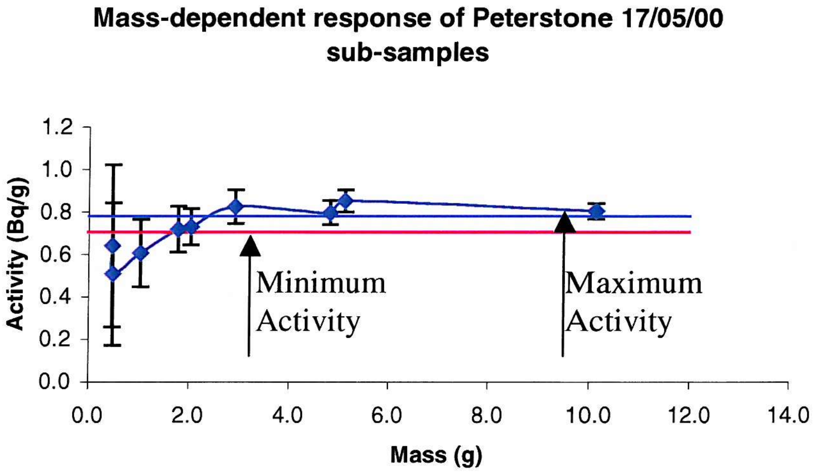


Figure 3.7: Mass-dependent response of a single sample

3.6 Organically Bound Tritium

The nature and thus the definition of OBT varies according to the system it is in. Exchangeable or labile OBT is tritium bound to an electronegative atom such as oxygen, nitrogen or sulfur and is thus removable by exchange reactions with the hydrogen atoms of water or polar solvents. This form will be in equilibrium with the aqueous component of the system (water exchangeable tritium). Stable or non-exchangeable OBT, is T bound to a carbon atom and is only released during decomposition, or in the presence of strong acids, strong bases or catalysts that result in the breaking of the C-T bond. For the purpose of this study, OBT will be defined as stable or non-exchangeable tritium, where the OBT measurements are derived from the difference between the total tritium values (measured by furnace combustion) and the water-extractable values obtained using the procedure described in Section 3.4. It must be borne in mind however, that the water extractable fraction refers to tritium that is leachable in water and therefore may contain other water-soluble species. Removing the water fraction (HTO) can also lead to distortion of results with both disruption of the OBT/HTO equilibrium and isostopic effects. [Ware & Allot 1999]

3.7 Summary

This chapter outlines the steps involved in the development of the analytical procedures employed for tritium measurements throughout this study. A method of analysis for OBT has been developed, where the working definition of OBT is the difference between total tritium (obtained from combustion of wet environmental samples under a controlled environment), and water-extractable tritium measurements. The limit of detection for total tritium measurements is 0.01-0.03 Bq/g at a 95% confidence level. This method of analysis is consistent with the recommendations made by Ware & Allot [1999] who suggested that the analysis of OBT should be based on calculating OBT by difference of total tritium and tritiated water.

3.8 References

Altshuler, B., Pasternack, B., Hlth. Phys., **9**, p.293, 1963.

Currie, L.A., *Limits for Qualitative Detection and Quantitative Determination,* Analytical Chemistry, **40**, p. 586-593, 1968.

NCRP, Tritium in the environment, Report no. 62, National Council on Radiation Protection and Measurements, Washington DC, 1979.

Ware, A., Allot, R.W., *Review of Methods for the Analysis of Total tritium and Organically Bound Tritium,* Report no. NCAS/TR/99/003, National Compliance and Assessment Service, Cameron House, Lancaster, 1999.

4.0 Tritium in Cardiff Bay

4.1 Introduction

The aim of this study was to determine the spatial and temporal variation of tritium in the Cardiff Bay area and to identify the forms of tritium present in sediment from that area. This involved the collection of surface sediment samples from 7 sampling sites in the Cardiff Bay area. The sampling site locations are shown in Figure 4.1. After collection the samples were analysed using the combustion technique outlined in Section 3.5.3.

Sampling in Cardiff Bay commenced in February 2000, and ended in February 2001. In total 84 samples were collected during 9 sampling trips. Monthly sampling was adhered to as much as possible, with sampling carried out during low tide. Sample collection at all sites was not always possible due to weather and tidal conditions. The outbreak of 'Foot and Mouth' disease also prevented some sample collection.

Sampling sites were chosen due to their location (relative to the sewer outpipe from Nycomed- Amersham), ease of accessibility and whether or not they had been used in previous research (eg. by AEA Technology). Sites were located and their positions recorded by GPS to within ± 50m. The same 'general' areas were sampled on each occasion. The site descriptions and locations are to be found in Appendix 1.0

1 - Barry Island
2 - Orchard Ledges SW
3 - Orchard Ledges NE
4 - Maerdy Farm
5 - Peterstone Great Wharf
6 - St Brides Wentlooge
7 - Gold Cliff

Figure 4.1 Map of the United Kingdom showing the location of Cardiff and Hinkley, and a map of the sampling locations in the Severn Estuary.

4.2 Sampling methodology

Surface scrape samples were taken from 7 sites along the Welsh coast of the Severn estuary (see Appendix 1 for site description). The sites sampled are shown in Figure 1.1, and were all accessible intertidal mudflat areas. Sampling was shore-based with scrapes being taken manually, with the aid of a plastic scoop, at low tide, on a monthly timescale. The surface sediments were taken from an area of 25 x 25cm to a thickness (depth) of 1 cm. The samples were taken back to the laboratory where they were subsequently archived before being frozen prior to analysis by the combustion technique outlined in Section 3.4.3.

4.2.1 Spatial variation of ^3H in Cardiff Bay Sediments

Tritium is discharged into the Cardiff Bay area in the form of tritiated water and organically bound tritium, from one discrete point. Movements of sediments due to the severe tidal and storm activity in the area will lead to its redistribution and settlement within specific areas. Measurements of total tritium activity in surface scrape samples of 15-20g were made and are shown in Figure 4.2.

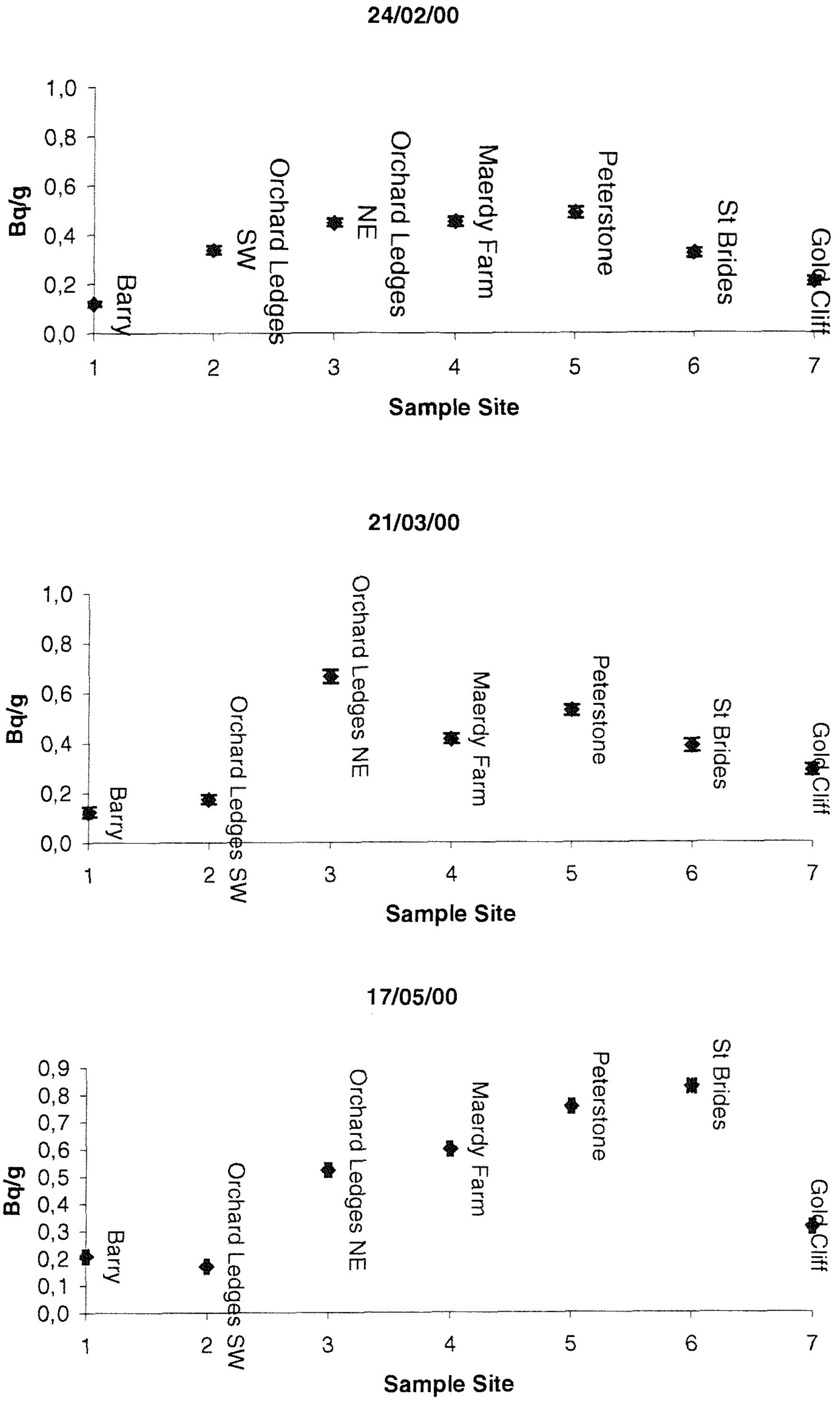
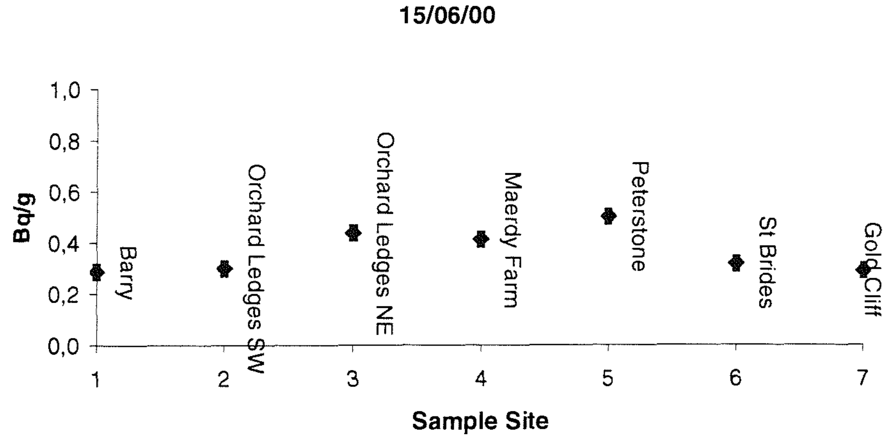
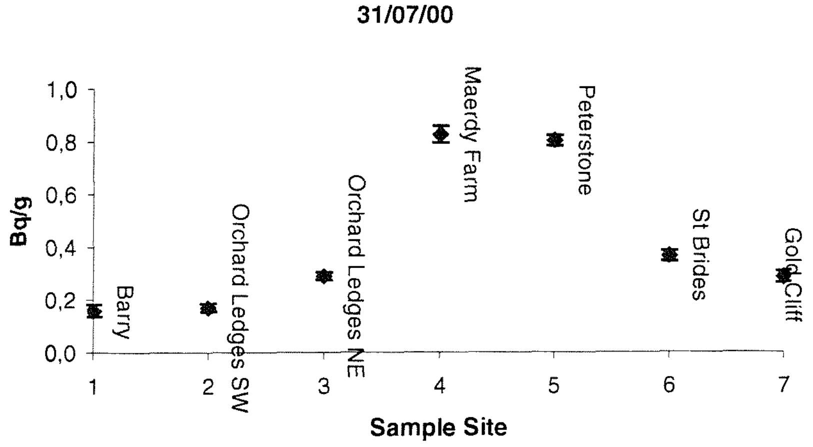


Figure 4.2: Total tritium activity in surface sediment samples in Cardiff Bay ± 2sd





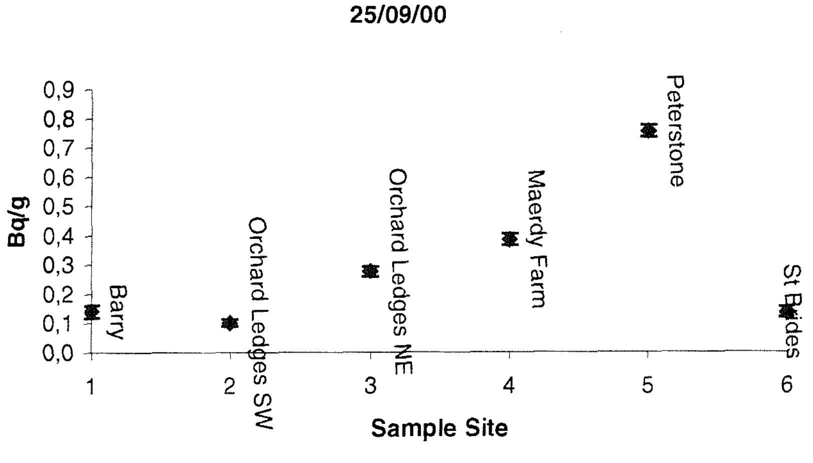


Figure 4.2 cont: Total tritium activity in surface sediment samples in Cardiff Bay ±2sd

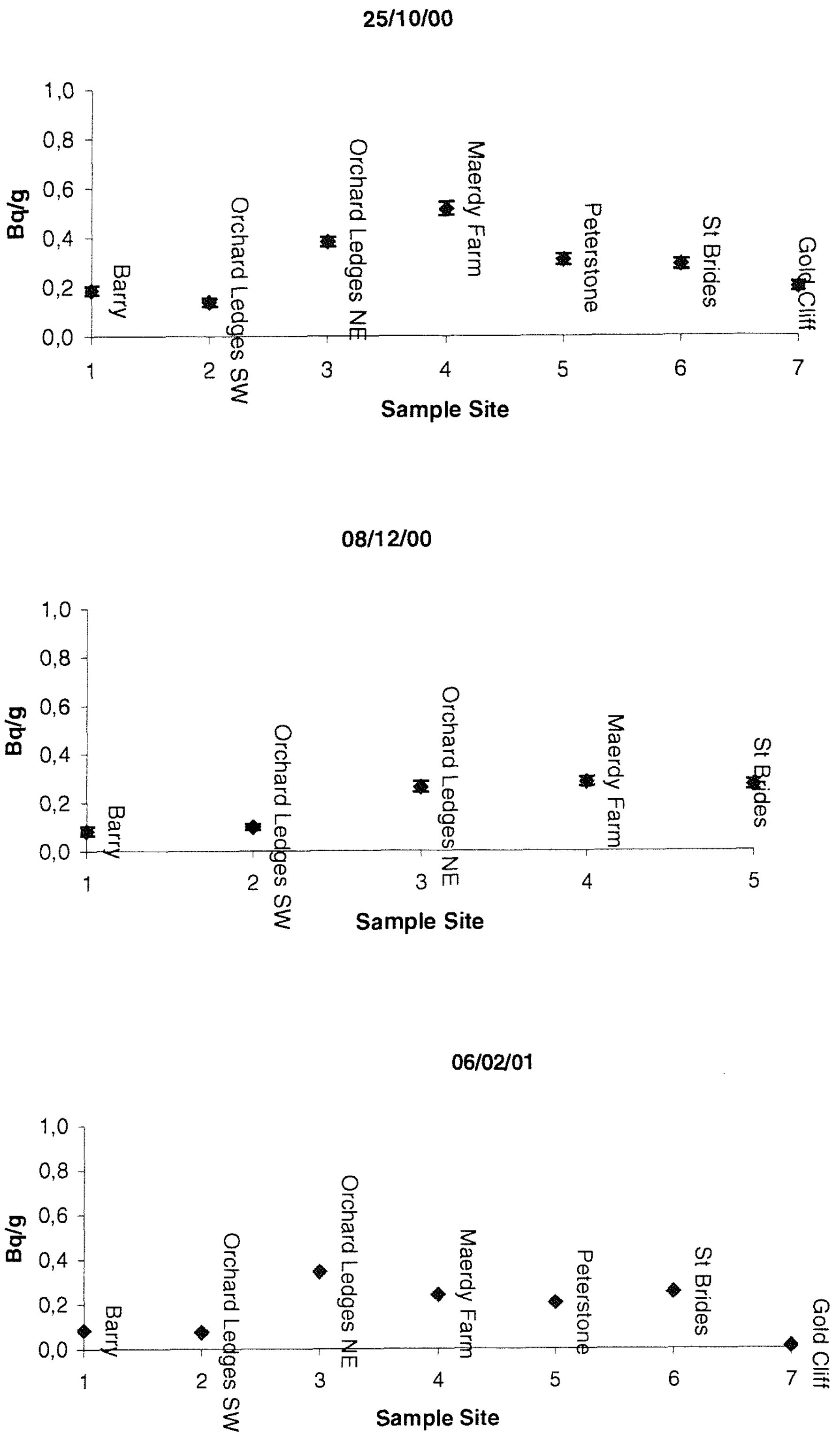


Figure 4.2 cont.: Total tritium activity in surface sediment samples in Cardiff Bay ± 2sd

4.2.2 Temporal Variation of ^3H in Cardiff Bay Sediments

Tritium is discharged into the Cardiff Bay area, in the form of tritiated water and organically bound tritium, from one discrete point. Severe tidal movements and storm activity in the winter months, and hot, dry spells in the summer, may have an affect on the distribution of surface sediments found in the area. Figure 4.3 illustrates the temporal variation of total tritium activity at each of the seven sites sampled.

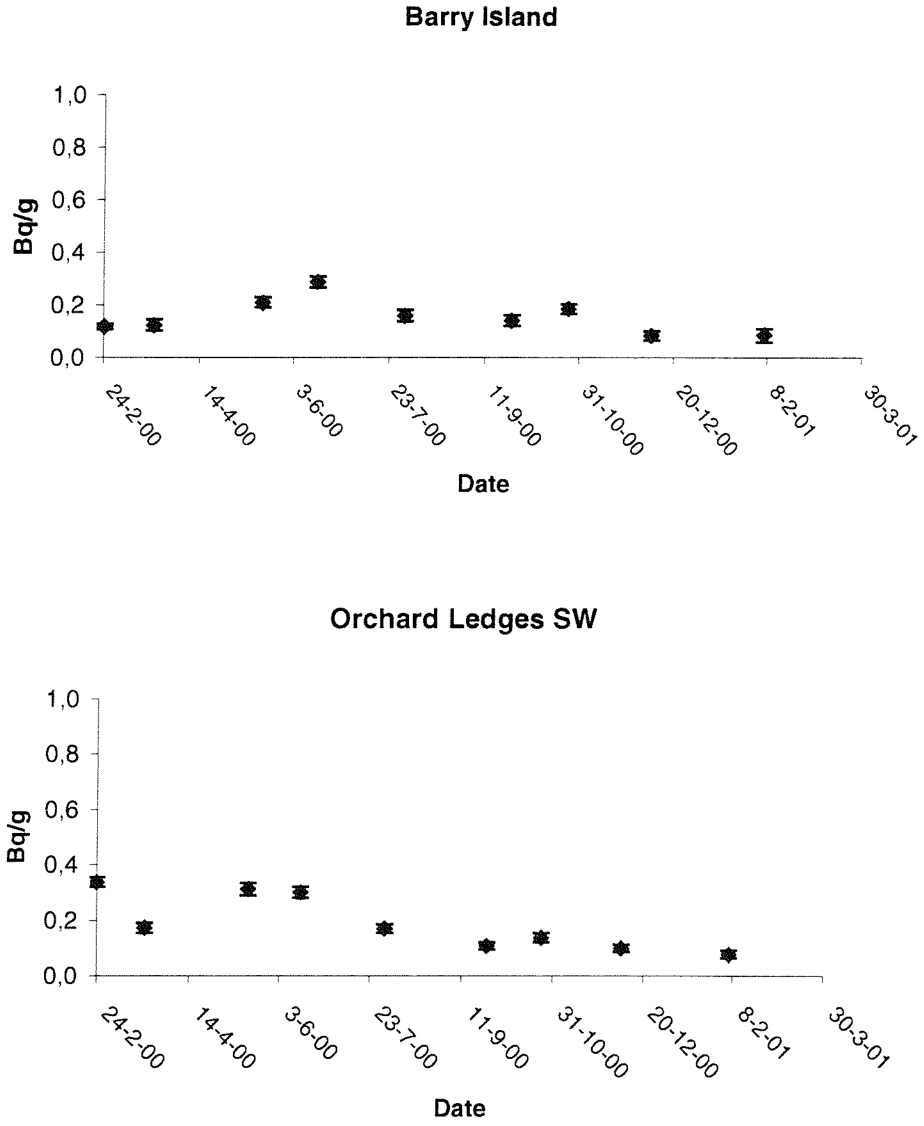
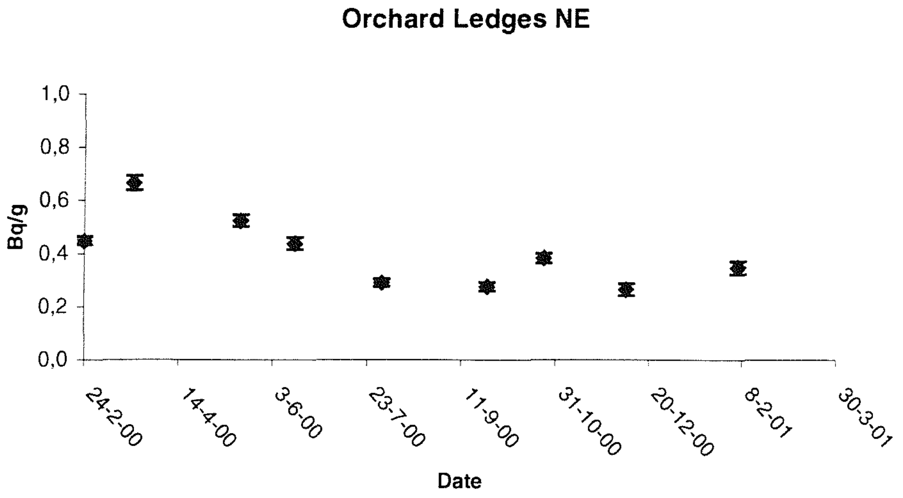
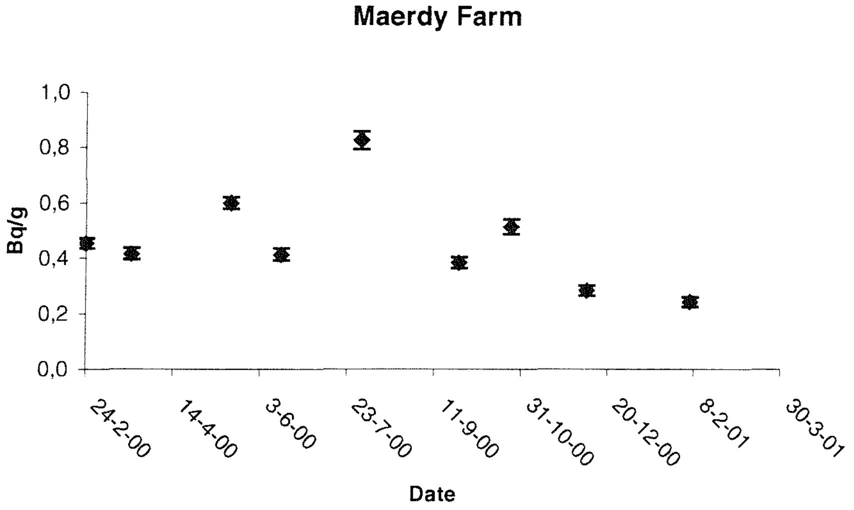


Figure 4.3: Temporal variations in total tritium activity of surface sediment samples. ± 2sd





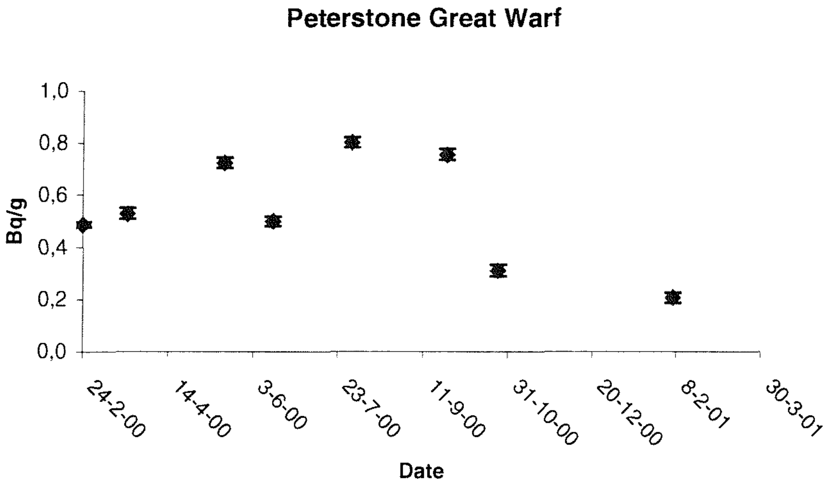
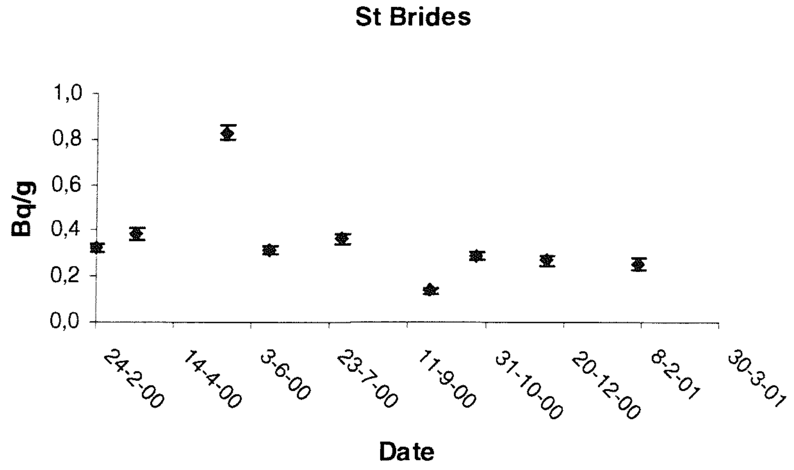


Figure 4.3 cont: Temporal variations in total tritium activity in surface sediment samples ± 2sd



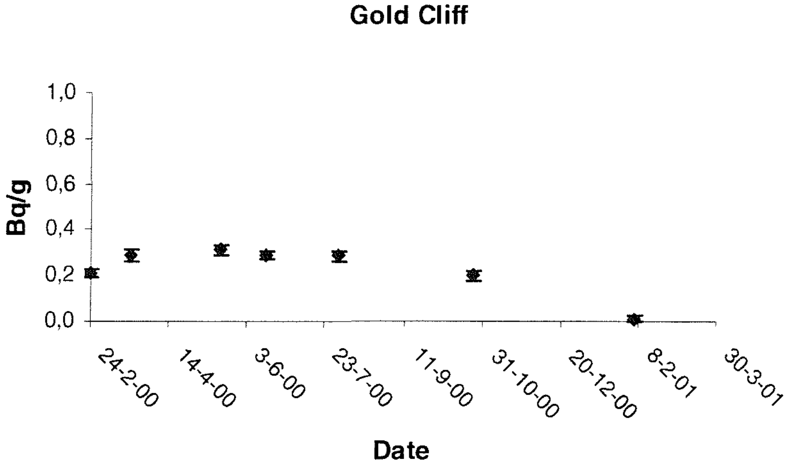


Figure 4.3 cont: Temporal variations in total tritium activity in surface sediment samples ± 2sd

4.2.3 Results and Discussion

Tritium activity is spread widely across the Cardiff Bay transect, but there is a general tendency for levels to reach a maximum in the Maerdy Farm- Peterstone area, before dropping off again towards Gold Cliff. The most contaminated area of the bay is not fixed and highest activities are found in any area from Orchard Ledges NE to St Brides. This is consistent with activity shifting position as a result of tidal and wind/wave action moving sediment between sites.

Despite the monthly variations, the lowest activities are consistently found at Barry Island and Gold Cliff sampling sites (Figure 4.3). These two sites are the furthest easterly and westerly sites from the discharge pipe. The activity at these sites shows little variation over time, with activities consistently around 0.2Bq/g for Barry and 0.2-0.3 Bq/g for Gold Cliff. Results obtained by McCubbin et al [2001] for Orchard Ledges showed activities in the fined grained muddy sediment of ~ 600 Bq/Kg. The values obtained from this survey were of the order of 200-400 Bq/Kg. Their data, although not exactly consistent with this survey, is within the same order of magnitude. The activities found at Orchard Ledges might be expected to be higher than other areas because it is the nearest accessible shoreline sampling location to the Cardiff sewer outfall which lies~ 2km offshore. [McCubbin et al 2001]

Higher activities are to be found in the sediments during the summer months than during the winter months. This is consistent with the purging of activity from the discharge site as a result of storm activity. Significant reductions of the sediment depth was observed during the winter sampling trips, supporting the hypothesis that sediment movements result in the movement of tritium activity away from the discharge site. During the winter months samples were generally coarser and sandier in nature compared to the summer samples. Allen and Duffy. [Sep. 1998] also found that their samples in this area were of a coarser/sandier nature in the winter months. The decrease in mud available for sampling may have a profound affect on the sample activity, as tritium activity in marine sediments is generally associated with the finer organic surface fraction of the sediment.

The Nycomed-Amersham discharge data for the year 2000 reveals a gradual decrease of tritiated waste from January to August, ranging from 9.25 x 10^{12} to 4.5 x 10^{12} Bq/g. The temporal activity trends shown in Figure 4.3 do not mirror this decrease in activity, although there does appear to be a general decrease in activity in the last few months of sampling. This decrease in activity, as mentioned previously may be due to storm activity, and as a result the surface mud may have moved elsewhere or be suspended in the water, and not the decline in activity released into the environment. It is difficult to justify however, why, in an area of such tidal activity, that the highest OBT measurements are consistently found in the same areas, where these areas are not necessarily the closest to the sewer outpipe. This may be explained by tidal currents in the area, and predominant wind/wave action directing the activity to certain sites.

4.3 Water-extractable Tritium

This study is concerned with the measurement of organically bound tritium (OBT), where OBT is defined as 'non-aqueous'tritium. In order to obtain OBT values from the Cardiff Bay sediment samples, the amount of tritium present as 'aqueous'or water-extractable tritium must first be determined, and then subtracted from the total tritium measurements already illustrated in Figures 4.2 and 4.3. The method used to determine water-extractable tritium measurements is outlined in Section 3.4 and measurements were carried out on all the surface sediment samples collected. The method used involves mixing a wet sample with a known amount of water, water-exchangeable tritium would equilibrate with the added water and could be subsequently measured using liquid scintillation techniques. This method allows the determination of the fraction of tritium present in the sample in the form of aqueous tritium.

4.3.1 Results and Discussion

The tritium discharged into the Cardiff Bay via the sewer outlet pipe is in the form of organically bound tritium and tritiated water. Water extractable tritium measurements were carried out on all the undried samples collected and used to help determine the form of tritium found in surface sediments in the area. Water-extractable tritium values are very low, often below the limit of detection or very near to it. This form of tritium is therefore only a small proportion of the tritium in the sediments. (Figure 4.4)

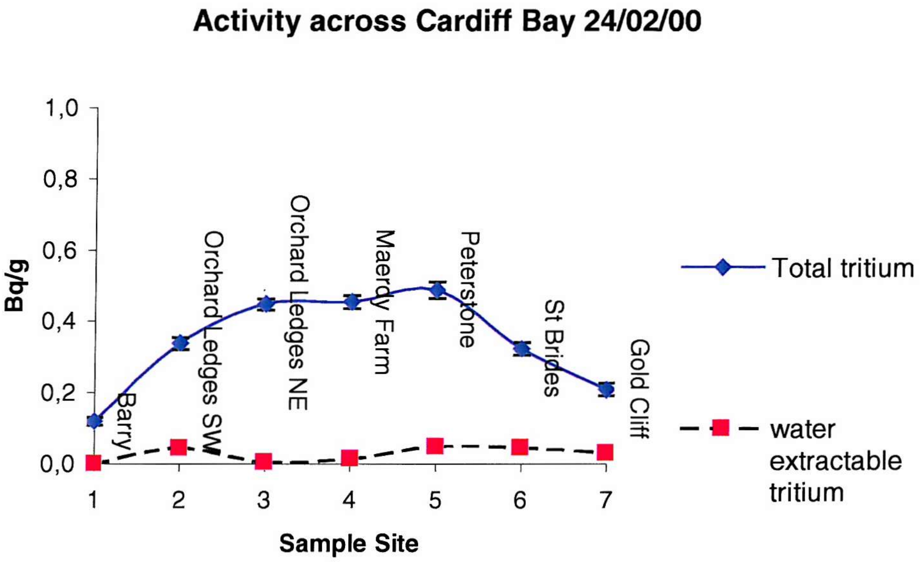


Figure 4.4: Water extractable and total tritium activity

The water extractable T values illustrated in Figure 4.4 are consistent with the remainder of the data from this study and with the findings of McCubbin et al. [2001], who found that the percentage of tritium, in the form of OBT, in sediments in this area was >85%.The majority of the measurements were below the limit of detection (0.01-0.03 Bq/g), and those that were not were often in the range 0.02-0.04 Bq/g, and hence were very near to it. Complete water extractable tritium results are shown in Tables 4.1 and 4.2. The results illustrate that there is very little, if any at all, water extractable tritium found in the surface sediment samples analysed, and that total tritium measurements are therefore representative of OBT, where OBT is operationally defined as the difference between total tritium and water-extractable tritium measurements.

Sample	*Sampling Date*	*Water Extractable T Bq/g*	*Error*
Maff/trit/16	24-2-00	<0.019	0.01
Maff/trit/17	24-2-00	0.046	0.01
Maff/trit/18	24-2-00	0.006	0.01
Maff/trit/19	24-2-00	0.015	0.01
Maff/trit/20	24-2-00	0.050	0.01
Maff/trit/21	24-2-00	0.047	0.01
Maff/trit/22	24-2-00	0.031	0.01
Maff/trit/23	21-3-00	<0.053	0.03
Maff/trit/24	21-3-00	0.024	0.03
Maff/trit/25	21-3-00	0.010	0.03
Maff/trit/26	21-3-00	0.010	0.03
Maff/trit/27	21-3-00	0.020	0.03
Maff/trit/28	21-3-00	0.047	0.03
Maff/trit/29	21-3-00	0.006	0.03
Maff/trit/30	17-5-00	0.001	0.03
Maff/trit/31	17-5-00	0.031	0.03
Maff/trit/32	17-5-00	<0.020	0.01
Maff/trit/33	17-5-00	<0.039	0.03
Maff/trit/34	17-5-00	0.017	0.02
Maff/trit/35	17-5-00	<0.037	0.03
Maff/trit/36	17-5-00	<0.033	0.02
Maff/trit/37	15-6-00	<0.034	0.03
Maff/trit/38	15-6-00	<0.034	0.03
Maff/trit/39	15-6-00	0.006	0.03
Maff/trit/40	15-6-00	<0.032	0.03
Maff/trit/41	15-6-00	0.010	0.03
Maff/trit/42	15-6-00	<0.035	0.03
Maff/trit/43	15-6-00	<0.035	0.03
Maff/trit/44	31-7-00	<0.037	0.03
Maff/trit/45	31-7-00	<0.034	0.02
Maff/trit/46	31-7-00	<0.037	0.03
Maff/trit/47	31-7-00	<0.031	0.02
Maff/trit/48	31-7-00	<0.032	0.02
Maff/trit/49	31-7-00	<0.033	0.03
Maff/trit/50	31-7-00	<0.037	0.03
Maff/trit/51	31-7-00	<0.028	0.02
Maff/trit/52	31-7-00	<0.032	0.03
Maff/trit/53	31-7-00	<0.032	0.03
Maff/trit/54	31-7-00	<0.036	0.03
Maff/trit/55	25-9-00	<0.036	0.03

Table 4.1: Water-extractable tritium results for all sediment samples collected in Cardiff Bay

Sample	*Sampling Date*	*Water Extractable T Bq/g*	*Error*
Maff/trit/56	25-9-00	<0.014	0.01
Maff/trit/57	25-9-00	0.011	0.03
Maff/trit/58	25-9-00	<0.030	0.02
Maff/trit/59	25-9-00	0.029	0.02
Maff/trit/60	25-9-00	0.037	0.03
Maff/trit/61	25-10-00	<0.041	0.03
Maff/trit/62	25-10-00	<0.045	0.03
Maff/trit/63	25-10-00	<0.048	0.03
Maff/trit/64	25-10-00	<0.034	0.02
Maff/trit/65	25-10-00	0.001	0.02
Maff/trit/66	25-10-00	<0.039	0.02
Maff/trit/67	25-10-00	<0.030	0.02
Maff/trit/73	8-12-00	<0.035	0.02
Maff/trit/74	8-12-00	0.001	0.02
Maff/trit/75	8-12-00	<0.023	0.02
Maff/trit/76	8-12-00	<0.028	0.02
Maff/trit/77	8-12-00	<0.036	0.02
Maff/trit/78	6-2-01	<0.040	0.08
Maff/trit/79	6-2-01	<0.041	0.03
Maff/trit/80	6-2-01	<0.040	0.02
Maff/trit/81	6-2-01	<0.031	0.02
Maff/trit/82	6-2-01	0.041	0.01
Maff/trit/83	6-2-01	0.028	0.02
Maff/trit/84	6-2-01	<0.043	0.03

Table 4.1 cont.: Water-extractable tritium results for all sediment samples collected in Cardiff Bay

4.4 Analysis of Freeze-dried samples

A series of freeze dried samples was analysed to confirm that HTO is only a very small proportion of the tritium in the Cardiff Bay samples. During the freeze drying of sediment samples volatile tritium compounds would be lost with the sublimed water. A comparison of the tritium contents of fresh and freeze dried sediment samples should therefore indicate whether a significant proportion of the of the T in Cardiff Bay sediments is in the form of volatile species, most notable of which is water. A freeze dried sample (ca. 5g) was accurately weighed and added to an alumina crucible and combusted using the method described in Section 3.5.3.

4.4.1 Results and Discussion

Little difference was observed in the tritium levels found in fresh and freeze dried samples, with the two measurements lying within 2SD of each other. (Figure 4.3) The small discrepancies in the results, may be due to experimental error, or slight inhomogeneity within the sample itself. These results support the findings of the water-extractable experiment, in suggesting that the tritium activity found in the Cardiff bay surface sediment samples appears to be in a non-extractable, non-aqueous form. In addition it appears that the tritium species present in the sediment have low volatility.

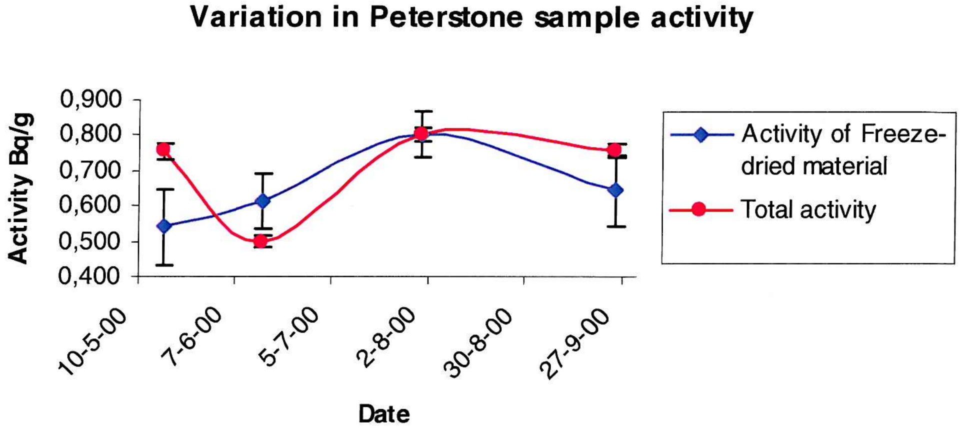


Figure 4.5: Variation in tritium activity for a series of fresh and freeze-dried samples

4.5 Conclusions

The analysis of surface sediment samples from Cardiff Bay, as shown in Figures 4.2 and 4.3, suggest that there are both seasonal and temporal variations in tritium activity measured in the area. A link between sediment composition and activity measured also seems apparent, with lower activities measured in coarser samples. Seasonality was the main influencing factor determining sample composition, which resulted mainly from variations in wind/wave, tidal and storm activity. The winter months tended to show increased storm activity, with a decrease in sample availability as a result. A possible explanation for the decrease in sample may be due to increases in tidal and wave action, resulting in more of the sample being present in suspension during sampling. The decrease in mud available for sampling appears to have had a profound affect on the sample activity, with evident decreases measured during periods of low sample availability.

High tritium activities were generally always found in the same areas, situated around the Maerdy Farm and Peterstone sites. Although the Orchard Ledges site is geographically closer to the sewer outlet pipe where the activity is discharged, predominant wind/wave action may be directing the activity and depositing it in these areas. These sites also appeared to show elevated levels at certain times of year when there was more fine, organic rich sediment available for sampling. This may suggest that tritium activity is related to the nature and composition of the sediment. Higher activities are generally associated with samples that were finer and more organic rich, whilst low activities tended to be measured when the samples were of a coarser, sandier nature. Variations in site activity were also evident, a possible result of fine sediment shifting position as a result of daily/monthly variations in the wind/wave and storm action.

Sections 4.3 and 4.4 illustrated that the predominant form of tritium found in the surface sediment samples analysed, was in the form of OBT, where OBT is defined as the difference between total tritium and water-extractable tritium. Tables 4.1, 4.2 and Figure 4.4 clearly show that water-extractable tritium measurements were near to or below the limit of detection (0.01-0.03 Bq/g), as determined in Section 3.5.6.The freeze-dried experiment supported these findings further by showing little to no variations in sample activity between fresh and freeze dried samples.

Although a number of conclusions have been drawn in this chapter, it must be noted that there were several limitations. Sampling in the Cardiff Bay region was not carried out at the exact time in the tidal cycle every month. Although a GPS was used it was only accurate to within 50m and therefore it is hard to guarantee that the exact same location was sampled every time. The outbreak of Foot and Mouth also meant that sample collection for a few sites was hindered.

4.6 References

Allen, J.R.L., Duffy, M.J., *Medium-term sedimentation on high intertidal mudflats and salt marshes in the Severn Estuary, SW Britain: the role of wind and tide*, Marine Geology, **150**, p. 1-27, Sep. 1998.

McCubbin, D., Leonard, K.S., Bailey, T.A., Williams, J., Tossels, P., Incorporation of Organic Tritium (^3H) by Marine Organisms and Sediment in the Severn Estuary/Bristol Channel (UK), Marine Pollution Bulletin, **42**, p.852-863, 2001.

5. Gamma Analysis of Samples

5.1 Introduction

The radionuclide content of Cardiff Bay sediments was examined in order to gain an insight into the mineralogy, redistribution and date of the sediments. All the samples were analysed by gamma spectrometry for ^{7}Be, ^{40}K, ^{137}Cs and ^{210}Pb. Subsamples of sediments were freeze-dried and then ground using a pestle and mortar. The activities were determined by counting subsamples using a Canberra 30% P-type HPGe gamma ray spectometer. The samples were counted using a constant geometry approach as opposed to constant mass, resulting in the content of subsamples varying in weight from 10-20g being counted, the method has a detection limit of 0.5Bq/g. A lead shield was placed around the detector to shield it from background radiation. All the spectra were analysed using a program called FITZPEAKS. The program locates peaks using a sensitive algorithm and then functions are used to fit peaks to the experimental data. [Cundy 1994] The instrument was calibrated for the sample geometry using the method described by Croudace [1991].

^{7}Be is a cosmogenic radionuclide (half-life = 53.29 days) which is continually deposited on the sediment surface. Its activity can be used to determine the mixing/reworking of surface sediments. ^{40}K activity is an indication of the mineral content of the sample. ^{137}Cs (half-life = 30 years) is present in the study area due to atmospheric fallout from nuclear weapons testing and reactor accidents as well as a result of authorised discharge from the nuclear processing plants. Marked maxima in deposition of ^{137}Cs occurred in 1958 and 1963/1964 (due to above-ground nuclear testing) and 1986 (from the Chernobyl accident). ^{210}Pb (half-life = 22 years) is a naturally occurring radionuclide formed as a product of the ^{238}U decay series. The measurement of ^{210}Pb is used to date recent (up to 120 years old) nearshore sediments of relatively high accumulation rate.

5.2 Results and Discussion

5.2.1 ^{40}K Results

^{40}K activity is an indication of the mineral content of the sample. The results shown in Figure 5.1 illustrate the peaks and troughs found in the activity of this radionuclide over time at the seven sites sampled in Cardiff Bay. Increases in activity are generally found when the quantities of fine, surface mud available for sampling were high. Troughs result from times when then fine sediments were absent and only coarse sand and debris quantities were present. Increases in sand and shell debris result in a dilution of the mineral content of the sediment.

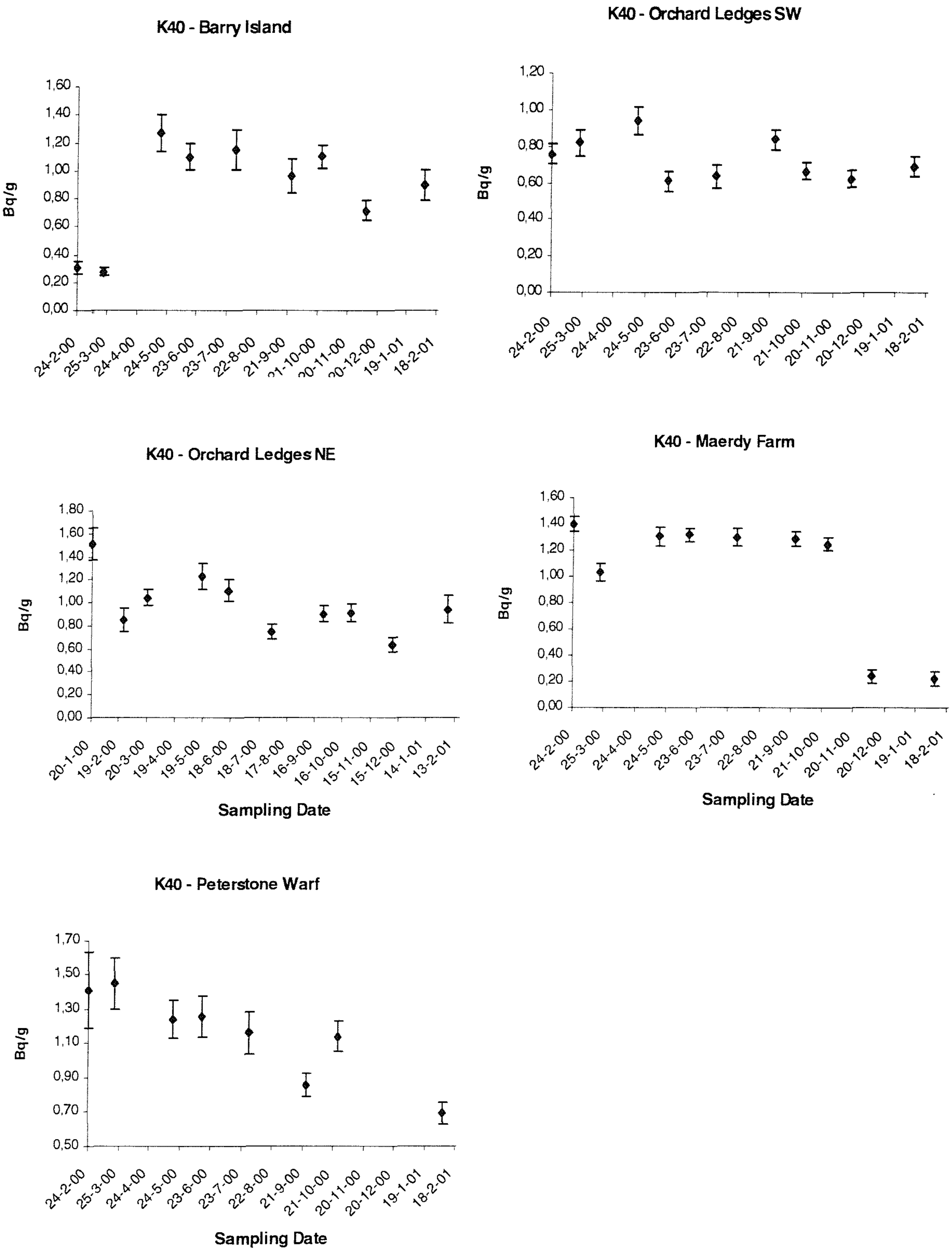


Figure 5.1: ^{40}K Variation over time ± 2 sd

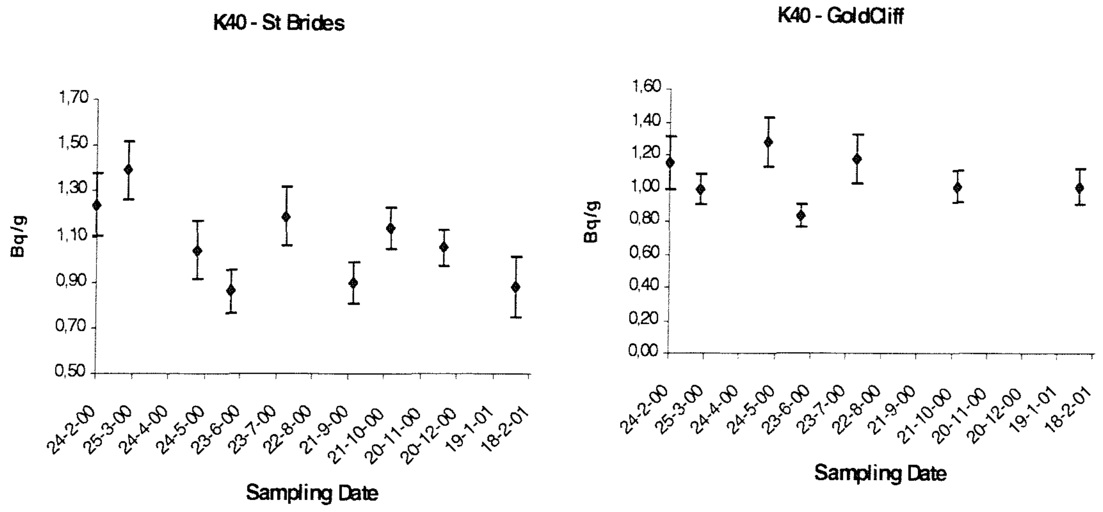


Figure 5.1: ^{40}K Variation over time ± 2 sd

5.2.2 ^{137}Cs Results

^{137}Cs has a strong tendency to associate with fine-grained sediments and can sorb strongly onto clay and organic particles. Figure 5.2 illustrates the results obtained from the Cardiff Bay subsamples. Peaks in activity may indicate the presence of clay-rich and organic rich materials, or may be the result of a tidal supply of labelled material eroding from a surrounding mudflat. A decrease in activity on the otherhand, may be due to mineralogical seffects, dilution by sands and coarse sediments or persistent mixing by the tidal regime. The nature of the environment also has an impact on the amount of this radionuclide that is present. In a marine/estuarine environment, ^{137}Cs is conservative in nature as it must compete with other ions in solution, mainly K^+ ions for available binding sites. Mudflat sites generally have low inventories of ^{137}Cs. [Cundy 1994]

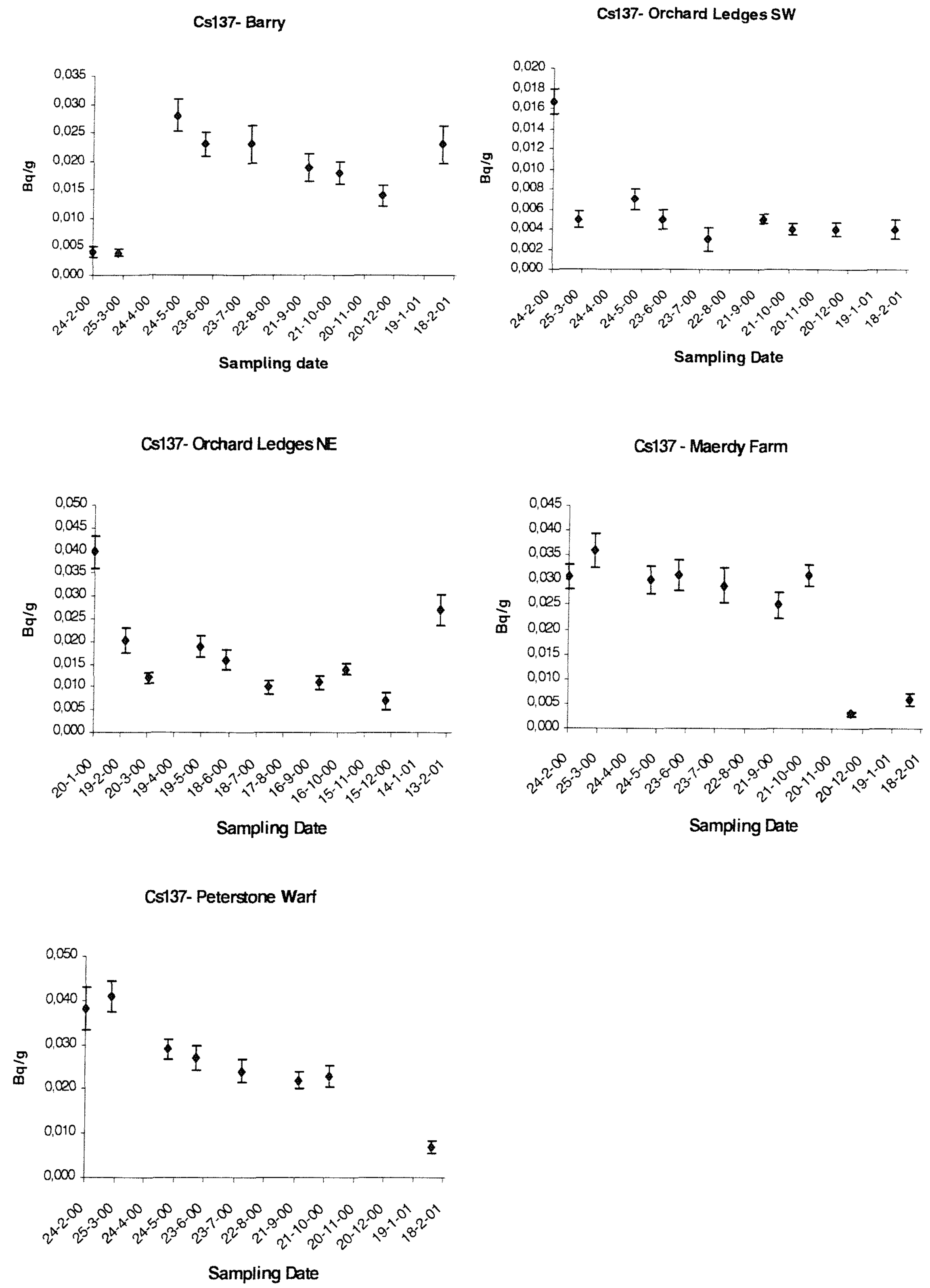


Figure 5.2: ^{137}Cs Variation over time ± 2sd

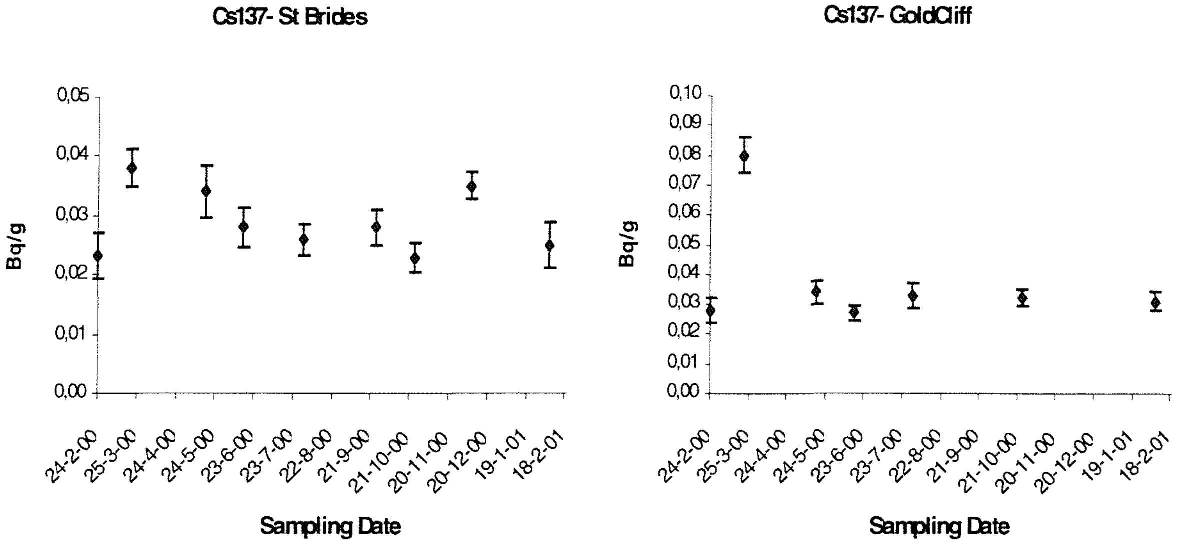
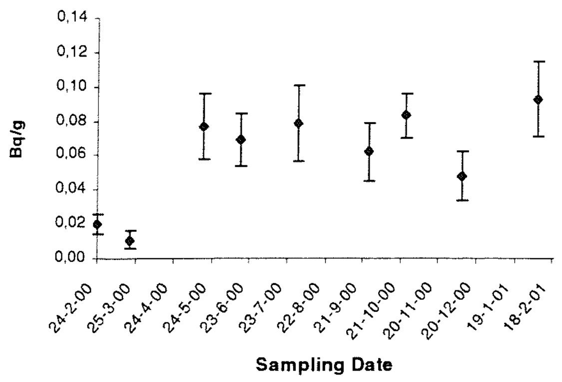
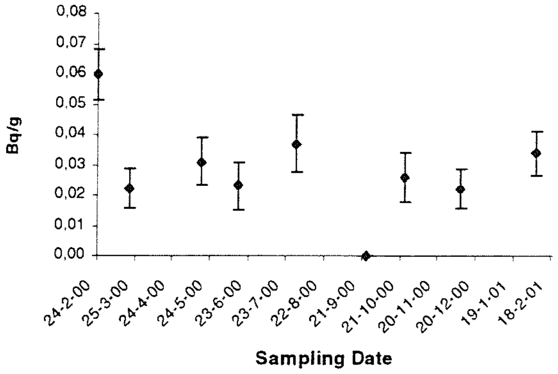


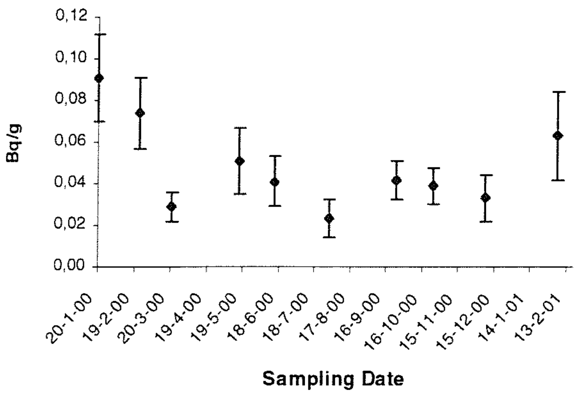
Figure 5.2: ^{137}Cs Variation over time ± 2sd

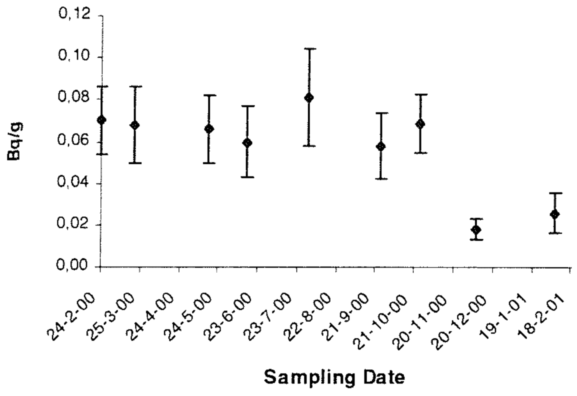
5.2.3 ^{210}Pb Results

^{210}Pb is a naturally occurring radionuclide formed as a result of the ^{238}U decay series. Measurements of this radionuclide provide a method of dating sediments up to 120 years old. The atmospheric input of ^{210}Pb is relatively constant throughout the year, with an expected atmospheric flux of 0.016 Bq/cm^2/yr and atmospheric fallout of 0.52 Bq/ cm^2. [Appleby and Oldfield 1992; Crozac et al 1964] ^{210}Pb also shows a preference for organic carbon, clay and Fe/Mn oxyhydroxide surfaces. This phenomenon may explain why the profiles of this radionuclide (shown in Figure 5.3) are very similar, and follow the same general pattern as ^{137}Cs, as illustrated by the profiles for Orchard Ledges NE, Barry, Maerdy Farm and Peterstone Warf. Minerogenic effects also affect ^{210}Pb distribution, with decreases in the inventories mimicking decreases in fine sediment (mud), as would be expected seeing as the radionuclide has a preference for organic carbon and clay surfaces as stated earlier


Pb210 - Barry


Pb210 - Orchard Ledges SW


Pb210 - Orchard Ledges NE


Pb210- Maerdy Farm

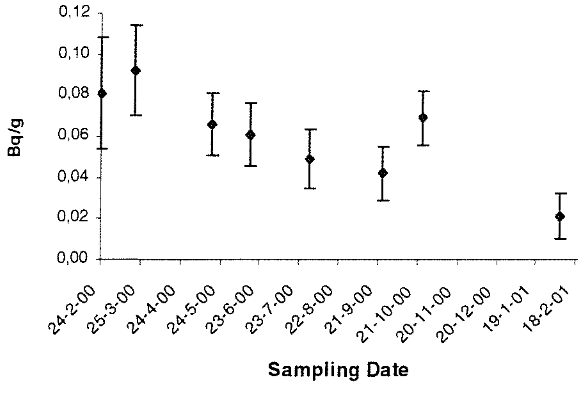

Pb210 - Peterstone Warf

Figure 5.3: ^{210}Pb Variation over time ± 2sd

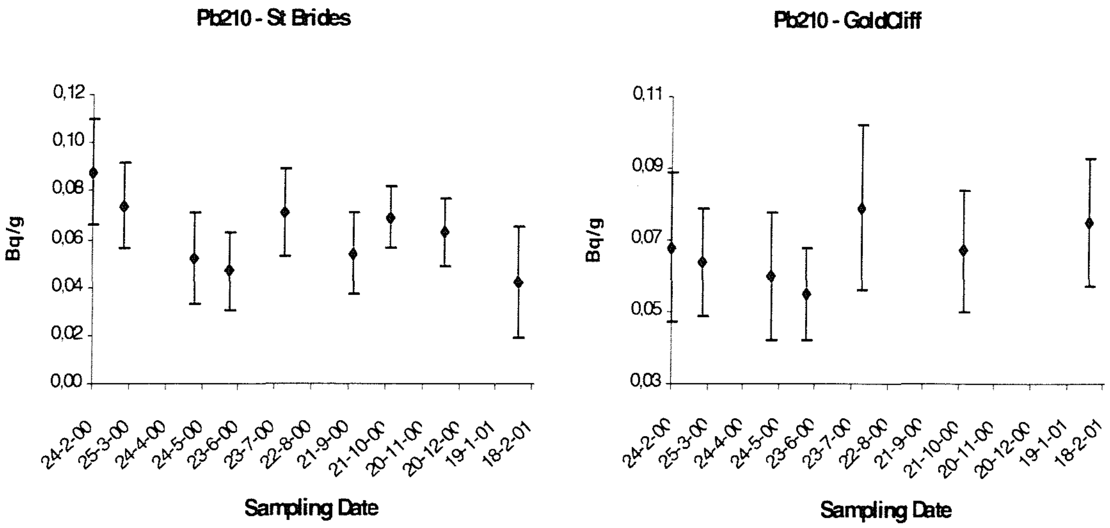


Figure 5.3: ^{210}Pb Variation over time ± 2sd

There are some noticeable trends shown in Figures 5.1, 5.2 and 5.3. Peaks and troughs in activity also tend to coincide with the quantity and type of sediment found at a location at a given time. Increases are generally associated with fine grained sediments (mud). Activities decrease at times when there is very little, or even no mud present, when only coarse sand and shell debris is present. Strong evidence for this decrease in activity is the Peterstone profiles, with very marked decreases in February 2001. In this month there was very little sampling material present, with only coarse sand and shell debris. In fact, there was a slow decrease in fine surface sediment (mud) available from Spring 2000- 2001, as illustrated by the gradual decreases in the charts. Maerdy farm also illustrates this phenomenon, with again very low inventories of all three radionuclides in December 2000 and February 2001, when there was only coarse sand and shell debris available for sampling.

5.3 Normalisation of Data

The concentrations of radionuclides in estuarine sediments are usually dependent on grain size and mineralogy, as a result of the association of metals with fine sediment particles. Normalisation removes spurious profile peaks, for example a clay rich sample may give rise to a peak in activity which cannot be related to a change in the input rate of the radionuclide. ^{137}Cs activity is normalised by dividing the activity by the ^{40}K content of the sample. This corrects for mineralogical variations that might arise in the sample.

5.3.1 Results and Discussion

The normalised ^{137}Cs data is shown in Figure 5.4. The charts generally illustrate increased activity with increased mud supply and a drop when the sediments are of a coarser/sandier nature. The drop in activity generally occurs during the winter months October-December when there is increased storm activity in this area, with subsequent increase in mudflat erosion. With the exception of St Brides, all sites appear to show little variation in activity between May and October. This may be due to reduced tidal activity and as a consequence dilution of the sediment. The lowest activities are generally found at Orchard Ledges SW, this is not entirely surprising as this site is the sandiest in nature. There is extreme tidal activity in this area and as a consequence a great deal of mixing and reworking of sediments.

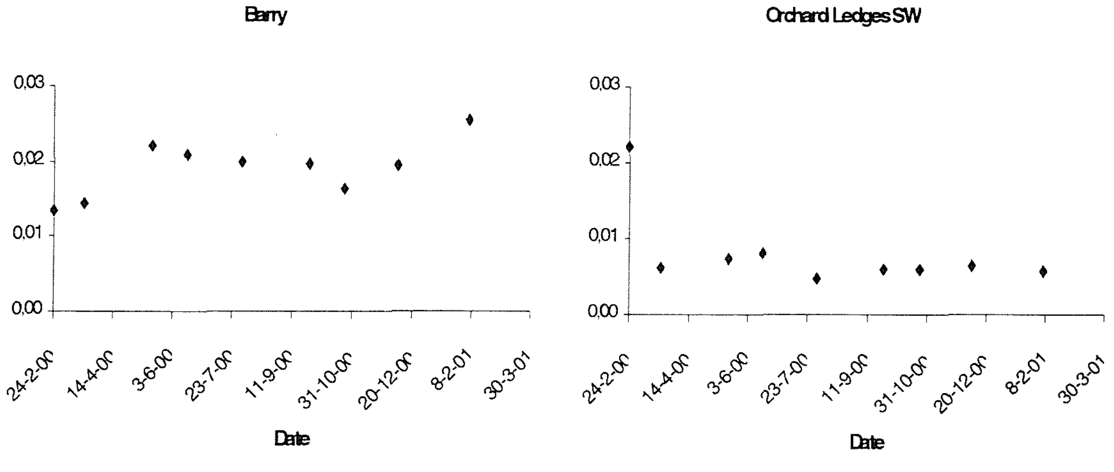


Figure 5.4: Normalised ^{137}Cs data ± 2sd

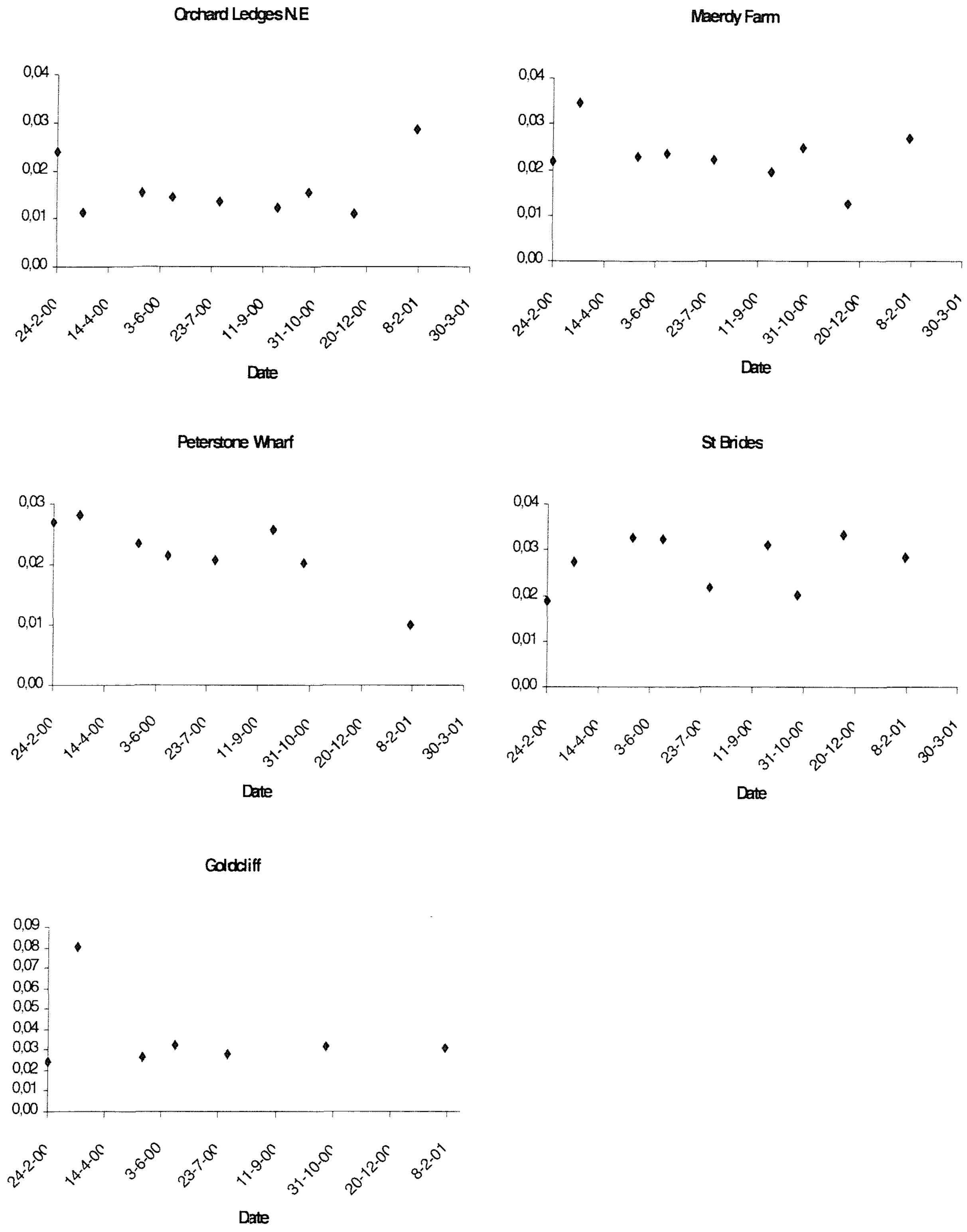


Figure 5.4 cont: Normalised ^{137}Cs data ± 2sd

5.4 Be-7 Activity

As stated in the introduction, ^{7}Be is a cosmogenic radionuclide, with a short half-life of 53.29 days. It is continuously deposited on the sediment surface and its activity can be used to determine mixing/reworking of surface sediments.

5.4.1 Results and Discussion

^{7}Be measurements were carried out on all the samples. Measurable activities were only found at a few sites, on a few occasions. Table 5.1 illustrates the ^{7}Be activities obtained and Figure 5.5 shows activities at Barry Island where ^{7}Be was found on several occasions.

Date	*Site*	*Be-7 Activity Bq/g*	*Error*
24/02/00	Maerdy Farm	0.0290	0.0120
25/09/00	Maerdy Farm	0.0180	0.0081
125/10/00	Maerdy Farm	0.0360	0.0083
17/05/00	Peterstone	0.0280	0.0150
25/10/00	Peterstone	0.0460	0.0150

Table 5.1: ^{7}Be activity at Cardiff bay sites

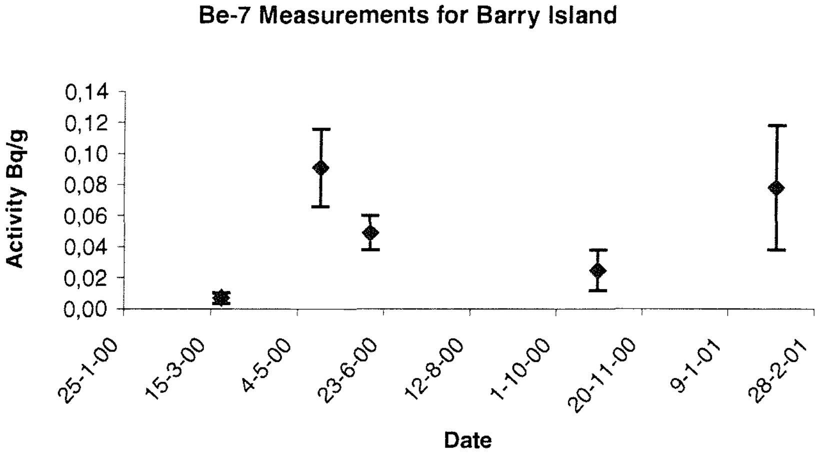


Figure 5.5: ^{7}Be activity over time at Barry Island ± 2sd

The ^{7}Be results are very important in determining the mixing/reworking of the sediments in the area of study. Apart from the results shown above, ^{7}Be measurements for all other samples were below the limit of detection ca. 0.01-0.03Bq/g. ^{7}Be has a very short half-life and is constantly deposited. It would be expected to be seen on sediments that were exposed and stationary. The lack of activity indicates therefore, that it is constantly diluted and reworked, a possible further indication that the study area is subject to constant mixing and reworking of sediments.

The presence of activity in the sites shown in Figure 5.5 and Table 5.1 is not entirely surprising. The Barry Island samples are taken from a harbour inlet which has a narrow connection to the open sea, roughly 50m wide. This area of mud is therefore fairly sheltered, and there is little reworking and mixing. The surface mud here varies with time and is slowly eroded; the trends for ^{7}Be at this site mimic those for ^{137}Cs. Peterstone and Maerdy Farm are however, much more open systems. Mud in these areas appears to be deposited in the spring and slowly erodes during the year, with maximum erosion during the winter months, possibly as a result of increased storm activity. The ^{7}Be present in the samples shown in Table 5.1, were taken after storm activities, when little mud was available. The sediment may not have been eroded by the storm or may be freshly deposited sediment.

5.5 Overall Conclusions

The different radionuclides analysed each provide information on the nature of the sediments found at the sites. From the data several conclusions can be drawn.

^{40}K is an indication of the mineral content of the samples. Increases in activity were generally found when the quantities of fine surface mud available were high, and troughs when only coarse sand and shell debris were found. Increases in sand result in the dilution of the mineral content of the sample.

^{137}Cs has a strong tendency to associate with fine grained sediment and can sorb strongly onto clay and organic particles. Levels of this nuclide were highest at times when the levels of fine grained mud were high and low when the amount of fine, organic-rich sediment was low. Peaks in activity represent the presence of clay-rich material or the influx of labelled material from the tidal supply. Decreases are due to increases of sand and coarse material in the area or due to persistent mixing by the tidal regime. Barry has high levels of this radionuclide due to its sheltered location, and levels at Orchard Ledges are low as it is a very sandy area. High activities are generally found in the Maerdy Farm- St Brides area due to the quantities of fine, clay rich sediment found in these areas,^{210}Pb results mimic those of ^{137}Cs, as the radionuclide has again a preference for organic carbon and clay materials. Peaks and troughs are representative of this fact, with increases found in times of high fine, muddy sediment availability and decreases when the sample was coarser and sandier in nature. The results of these three nuclides support the tritium measurements, with decreases in the concentrations mimicking decreases in tritium activity. This finding could suggest that the decline in tritium activity during the winter months is due to mineralogical affects, as the samples are generally coarser in nature.

The ^{7}Be results indicate that Cardiff Bay has high tidal activity and that there is constant mixing and reworking of the sediments.

5.6 References

Appleby, P.G., Oldfield, F., *Application of ^{210}Pb to sedimentation studies.* In Ivanovich, M., and Harmon, R.S., (Eds) Uranium-series disequilibrium. In Applications to Earth, marine and environmental sciences. 2nd Ed. Oxford Science, 1992.

Croudace, I.W., *A reliable and accurate procedure for preparing low-activity efficiency calibration standards for germanium gamma-ray spectrometers.* J. Radioanalytical Nuclear Chemistry Letters, **153**, p. 151-162, 1991.

Crozaz, G., Picciotto, E., and DeBreuk, W., *Antartic snow chronology with ^{210}Pb,* J. Geophysical. Research, **69**, p.2597-2604, 1964.

Cundy, A.B., *Radionuclide and Geochemical studies of recent sediments from the solent estuarine system.* Unpublished PhD thesis, University of Southampton.1994.

6. Partitioning Experiments

6.1 Introduction

Results from previous experiments suggested that the OBT found in Cardiff bay was associated with organic, clay-rich materials. Partitioning experiments were therefore used to assess the affinity of tritiated compounds for a variety of sediment components. A variety of clay materials and sediments were employed, equilibrated with tritiated compounds dissolved in solvents of varying polarity. As in the experiment outlined in Section 3.5.5, 7 tritiated compound groups were used to represent components of the Nycomed- Amersham waste.

6.2 Definition of a distribution (partition) coefficient

Two terms are employed to describe the distribution of a solute between 2 phases, distribution coefficient and partition coefficient. When a compound distributes itself between an aqueous phase and a solid phase (such as sea water and sediment) the distribution is defined by the concentration of the compound on the solid phase relative to that in the aqueous phase. It is not uncommon for a compound to dimerize or dissociate to some degree in either or both phases. This is measured as the **distribution coefficient, K_D**, which describes the relative affinity of a substance (A) for the 2 phases and is the ratio of the total concentrations in the two phases. The distribution coefficient is determined experimentally by mixing a known mass of non- aqueous phase with a known mass of aqueous phase containing the component under investigation. The mixture is shaken to achieve equilibrium and the amount of the component in either phase is determined. The K_D is then calculated using the following equation:

$A_{aq} + S \Leftrightarrow A_s + Aq$ where S and Aq are the solid and aqueous phases

$$K_D = \frac{[A]_s}{[A]_{aq}}$$

where $[A]_s =$ total concentration of all forms of A in solid phase

$[A]_{aq} =$ total concentration of all forms of A in aqueous phase

$$K_D = \frac{(A_{solid} \times M_{aq})}{(A_{aq} \times M_{solid})}$$

where A_{solid} and A_{aq} are the activities in the solid and aqueous phases, and M_{solid} is the mass of the solid phase and M_{aq} is the mass of the aqueous phase.

Separation by solvent extraction depends on the differences in solubilities of a substance in two immiscible solvents. The technique involves the constant contact of a solution (usually aqueous), with a second solvent (usually organic). Complete quantitative extraction of a solute into one of the layers is often the goal of such experiments but the extraction may not be quantitative. A component will partition between the two phases, with only a certain percentage extracted. A solute which is soluble in both phases will distribute between the two phases in a definite proportion. The amount extracted depends on the nature of the extractant and on the volumes of the phases. The *partition coefficient* **Kp**, for a species A is the thermodynamic equilibrium constant for the distribution of A between the two phases. Suppose species A is distributed between 2 immiscible phases a & b, the Nernst distribution or partition law states that, provided its molecular state is the same in both liquids and that the temperature remains constant the resulting equilibrium may be written as:

A (in phase a) ⇔ A (in phase b)

$$= \frac{Concentration\ of\ solute\ in\ solvent\ a}{Concentration\ of\ solute\ in\ solvent\ b} \qquad = \frac{[A]_a}{[A]_b} \qquad = K_P$$

Equilibrium is attained when the free energy of the solute is the same in each phase. Ideally, the ratio of activities for A in the two phases will be constant and independent of the total quantity of A. The degree of partitioning between an aqueous an organic phase is defined as the **partitioning coefficient, K_P.**

$$K_p = \frac{(A_{org})}{(A_{aq})}$$

where $(A)_{org}$ and $(A)_{aq}$ are the concentrations of the analyte at equilibrium, of the organic and aqueous phases. Generally, the numerical value for K_P approximates the ratio of solubility of A in each solvent. For ideal solutions, the partition coefficient of a substance between 2 phases is related to the free energy required to transport one mole of the substance from one phase to another. Up to a certain point, where one or both phases becomes saturated with a given solute, the partition coefficient is independent of the total actual concentration of solute in each phase. Partitioning coefficients are roughly a measure of the relative solubilities of a particular species in each case and are often estimated using solubility data. If A is an undissociated monomer in both phases, then $K_D = K_P$

6.3 K_D experiments with standards in seawater

6.3.1 Introduction

The distribution coefficients of tritiated organic compounds between sea water and model sediments was investigated. The sediments studied included a dried marine sediment, fresh sediment collected from the estuary of the river Itchen Southampton, a suspended (Poole harbour) sediment water sample with varying particle loadings, and an inert sand suspension in water. Sea water was used in order to be representative of the Cardiff Bay area.

6.3.2 Methodology

0.1g of sediment was added to a 10ml volume of sea water in a 22ml polyethylene vial, and a spike of tritium standard was then added using a pipette. For the suspension solutions, the solution was made up using a known mass of sediment in a litre of seawater, kept in suspension using a magnetic stirrer. A 10ml volume of this solution was then pipetted out and added to a 22ml polythene vial. Samples were mixed for 1 hour on a mixing wheel and then filtered using a Cyclopore track etched membrane filter (pore size 0.4μm). A 2ml aliquot of the seawater sample was added to a 22ml polyethylene vial and made up to 20ml with Goldstar scintillant. The counting efficiency for seawater was calculated (see Appendix 2 for details). The experiments were normally repeated in duplicate or triplicate.

Sediments used:	Fresh sediment, Itchen Southampton (A&B)
	Marine dried sediment- Exeter (Topsham)
	Poole harbour suspended solution, particle loading ca.1000mg/l
	Sand suspended solution, particle loading ca.400mg/l

6.3.3 Results and Discussion

The seven tritiated standards were spiked into a range of sediment/sea water mixtures (fresh sediment A & B were both collected at Itchen, Southampton but are 2 separate samples, where a sample appears twice the experiment has been done in duplicate) in order to try and understand how the standards would partition in a marine environment like Cardiff Bay. The distribution coefficients for the standards in each of these mixtures was calculated. The results are shown in figure 6.1.

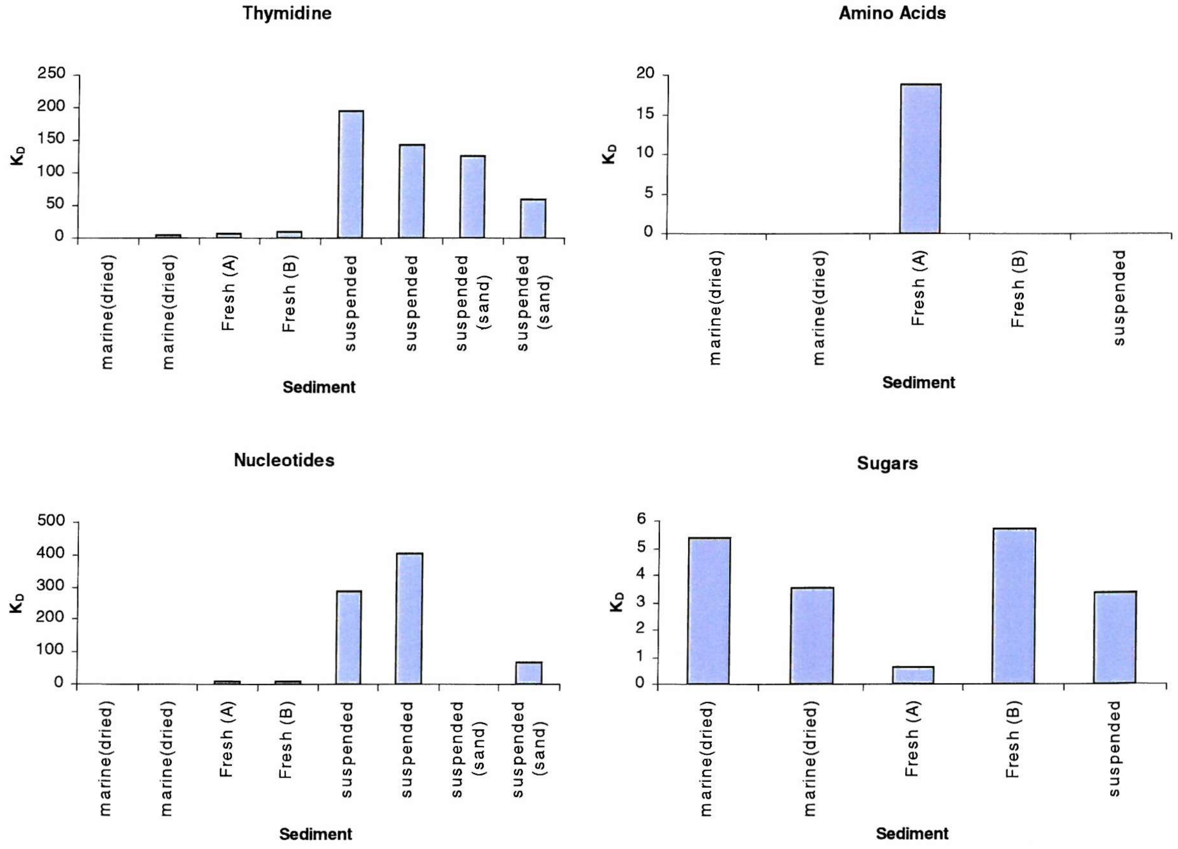


Figure 6.1: K_D values for tritiated standards and sediments

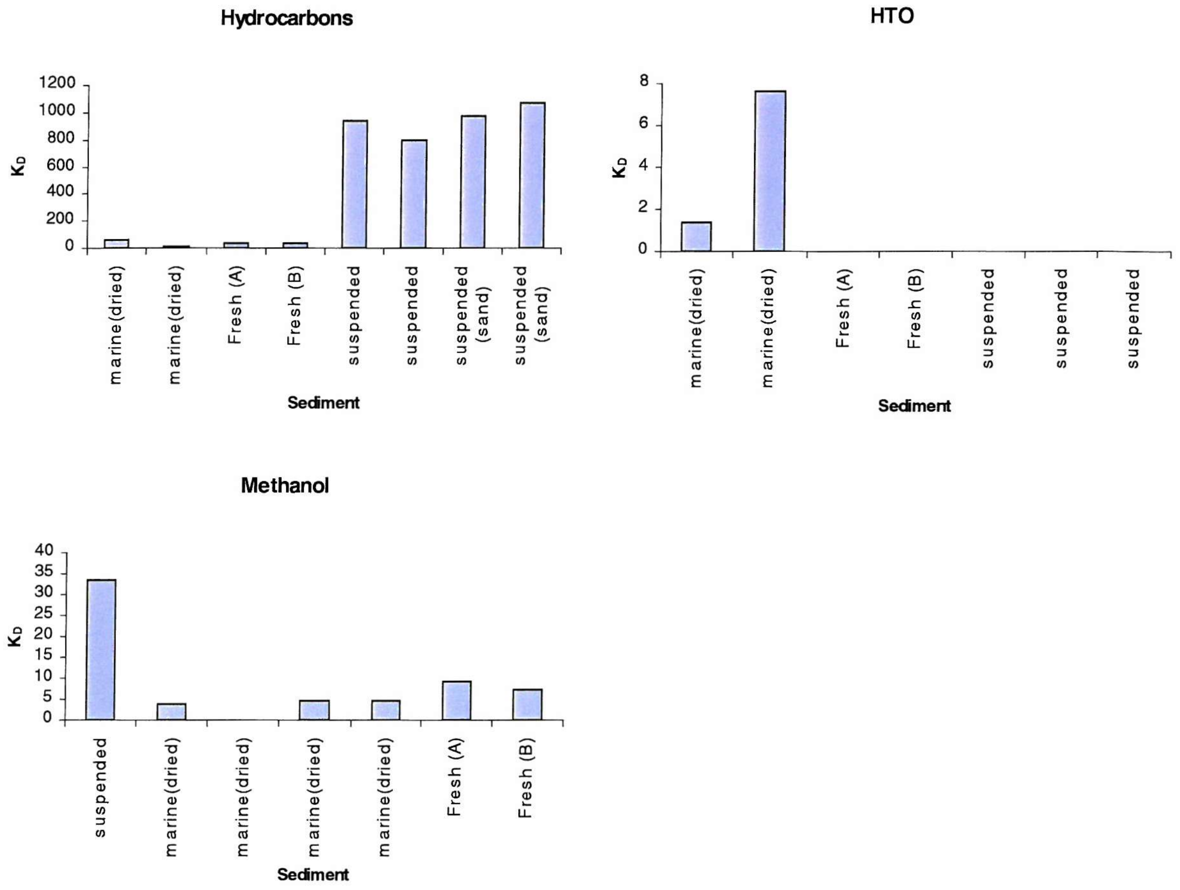


Figure 6.1 cont.: K_D values for tritiated standards and sediments.

The results from the partioning experiments with sea water, shown in Figure 6.1 show very little consistency. The magnitude of the distribution coefficient K_D varies from approximately 1000 for the hydrocarbon standard to values close to 0 for the amino acids, sugars, nucleotides, methanol, thymidine and HTO standards. The highest K_D values were generally obtained with the suspended solutions, a suspended solution in this experiment was made up of a known mass of dried sample (sediment) in a given volume of water. The mixture was kept in suspension by using a magnetic stirrer. The high values obtained for the suspended solutions may be due to the high surface area of these sediments. They may also be due to the nature of the sediment used. The Poole harbour sediment is organic rich and has an increased affinity for hydrocarbons and hydrophobic compounds. The marine dried sediment has the lowest K_D values with a maximum of approximately 5. This would indicate that the tritiated organics had a relatively poor affinity for sediment. The fresh sediments also have very low distribution coefficients, with values ranging from approximately 5-10.

6.4 Partitioning experiments with minerals and clays

6.4.1 Introduction

A series of clays, representative of those found in the Severn Estuary, were chosen and used in a number of partition experiments with tritiated standards. Kaolinite and montmorillonite (a smectite) were used as clays representative of the estuary, and calcite was used because it is a component of sedimentary rocks found in the area. The Severn Estuary has a high concentration of illites (micaceous minerals present in the clay-sized fractions of sedimentary rocks) and expandable clays (smectites). Generally, estuarine conditions favour the deposition of illite and kaolinite and disfavour smectite. Smectite settles less rapidly than other clay minerals, due to its frequent small size, flaky shape and low density. [Chamley 1989]

Key properties of clays are the uptake of cations by adsorption, ion exchange, interlayer absorption and structural replacement. The structural replacement, of e.g Mg^{2+} for Al^{3+} leaves a net charge, which in conjunction with the ionisation of silanol proups, Si-OH to $Si\text{-}O^-$ provides adsorption sites.[Pytkowicz 1983]. Kaolinite is a triclinic and well-ordered dioctahedral clay mineral, whose crystals often show distinct hexagonal outlines. It mainly forms in surficial environments through pedogenetic processes, but may also develop during early diagenesis. Montmorillonite belongs to the 'smectite group' whose characteristics determine the weakness of the linkage between the different layers of a given particle, and allows for considerable swelling. Illites represent the transition between montmorrilonite and the nonclay mineral mica; they are present in the clay-sized fractions of sedimentary rocks. [Chamley, 1989]

6.4.2 Methodology

0.1g of sediment was added to a 10ml volume of sea water in a 22ml polyethylene vial, and a 100μl spike of tritium standard (~ 3700Bq) was subsequently added using a pipette. Samples were gently mixed for 1 hour on a mixing wheel and subsequently filtered using a Cyclopore track etched membrane filter (pore size 0.4μm). A 2ml aliquot was taken from the seawater sample and added to a 22ml polyethylene vial, made up to 20ml with Goldstar scintillant and counted.

6.4.3 Results and Discussion

Clays and calcite, compounds representative of sediments found in the Cardiff Bay area were used in a series of partitioning experiments with 7 tritiated standards representative of Amersham-Nycomed waste. The results of this experiment are illustrated in Figure 6.2.

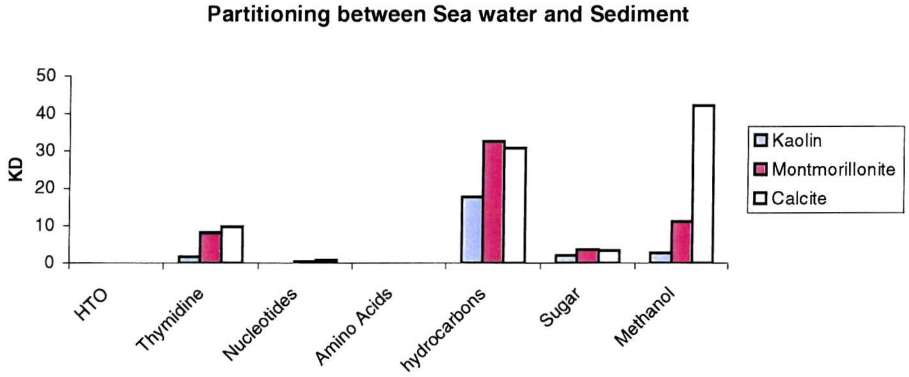


Figure 6.2: K_d's for standards in a sea water/ sediment mix

The distribution coefficients in Figure 6.2, ranged from approximately 0 to 50. Hydrocarbons had the greatest affinity for the clay samples, and there was some uptake of the methanol, sugar and nucleotide standards. Montmorillonite generally showed the biggest uptake of the sediments used, although methanol partitioned better on calcite. The standards used showed lowest affinity for kaolin.

Hydrocarbons showed the greatest overall affinity for the sediment phase. This may be due to the hydrophobic, non-polar nature of the aromatic compounds (dimethylbenzanthracene and benzopyrene) used in the experiment. Partitioning of such aromatics onto the clay surface may however better represent the poor solubility of these compounds in water, than a specific affinity of these compounds for clays. Higher surface areas may also contribute to the high K_D values.

There was no measurable uptake of amino acids onto the mineral fractions. The fact that there was little adsorption may be due to the pH of the seawater affecting the amino acids' overall charges. All the amino acids had neutral or basic side chains, and therefore would be anionic in nature in seawater (pH ~8), with the exception of lysine which would be zwitterionic at this pH. All the amino acids therefore would be ionized to some extent and hydrophilic in nature, hence the low affinity for the clay surface. Clays have a cation-exchange capacity under the right conditions, and hence other charged species possibly present in the sea water fraction may be competing with the amino acids for binding sites.

Sugars, nucleotides and methanol are all polar and therefore hydrophilic species. Their low K_D's may be attributable to the fact that they remain in the seawater fraction, as there would be a large loss of electrostatic solvation energy if they were to partition onto the sediments. The methanol in water fraction showed a high K_D value with calcite. This may be due to the nature of the methanol waste compounds. The exact composition of which was unknown.

6.5 Partitioning of OBT between water and organic solvents

6.5.1 Introduction

The partitioning of six Amersham tritiated standards between water and organic solvents, was assessed using hexane, toluene and dichloromethane (DCM). These solvents were chosen to cover a wide polarity range, with increasing polarity from hexane to DCM. After equilibration of the tritiated standards between the two phases, an aliquot of the water fraction was removed and counted using liquid scintillation counting. The toluene fraction of one experiment was counted in conjunction with the water sample to ensure that all the standard added could be accounted for. The standard was added as a spike and subsequently weighed. The activity added to the mixture was calculated and using scintillation counting the amount in the water sample counted was measured as a percentage of the added tritium.

6.5.2 Methodology

10ml of solvent was added to 10ml of sea water in a 22ml glass vial.The vial was then weighed and the mass noted. A 100µl spike of tritiated sugar standard was added and the vial reweighed in order to measure the exact amount of activity added. Samples were mixed for 1 hour on a mixing wheel, and 2ml of the sea water was pipetted out into a 22ml polyethylene vial for counting. An additional experiment was carried out with toluene in which a 5ml volume of toluene was also counted using liquid scintillation counting.

6.5.3 Results and Discussion

Partitioning experiments with solvents of increasing polarity, were carried out in order to measure the affinity of representative tritiated compounds for different media. In order to ensure that all the trititated activity added was accounted for, both the toluene and water fraction of the toluene/sea water experiment were measured. The results are shown in Figure 6.3.

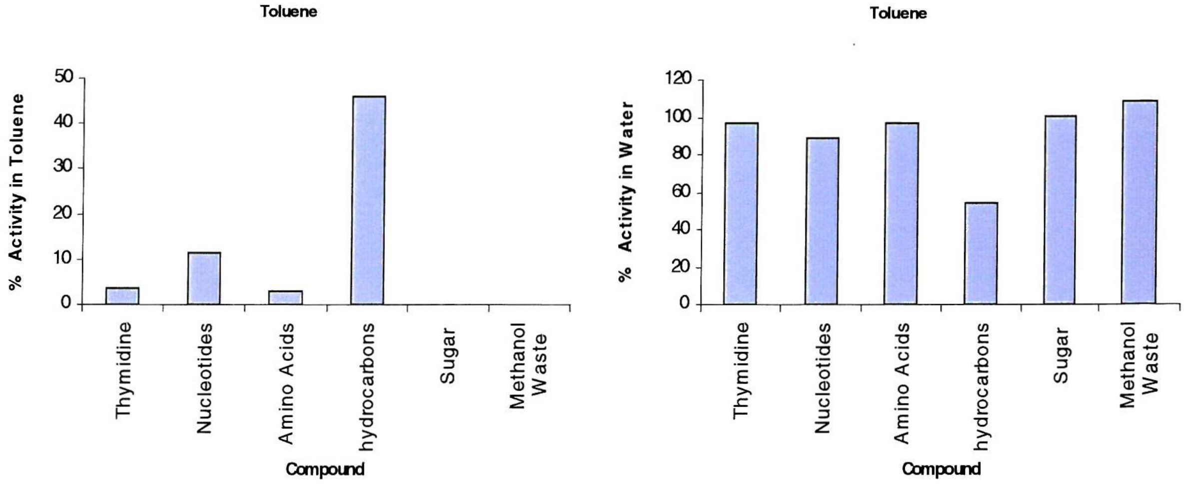


Figure 6.3: Partitioning of tritiated compounds between toluene and sea water.

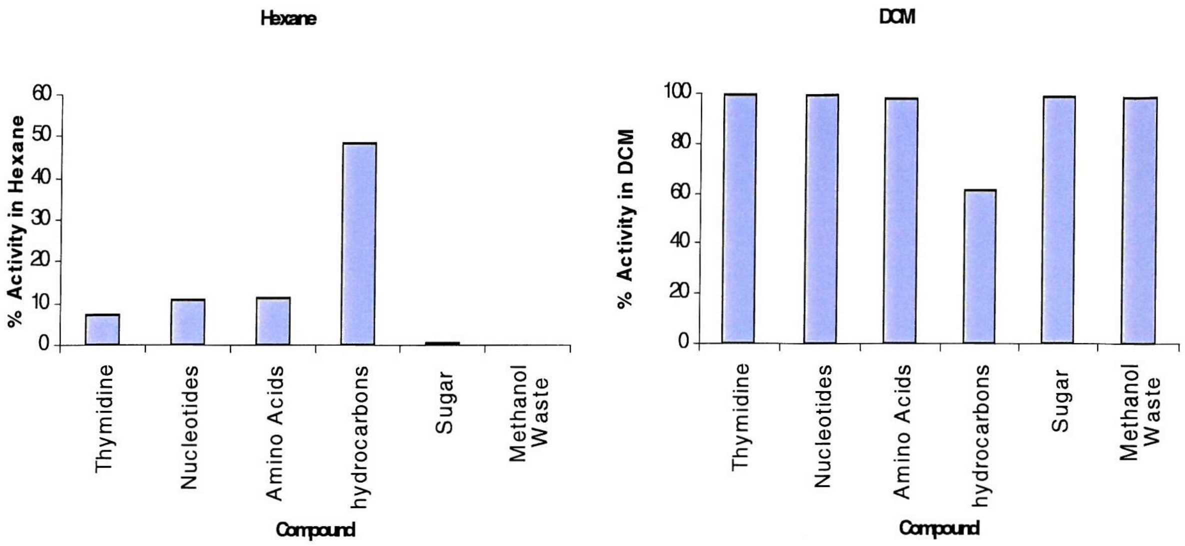


Figure 6.4 : Partitioning of tritiated compounds between organic solvents and sea water.

The results of the toluene experiment illustrate that close to 100% of the 6 standards added is accounted for, with the % extraction values for both the toluene and water fraction counted, adding up to ~100% (the slight loss may be due to counting efficiencies). The majority of the standards added into toluene, with the exception of the hydrocarbons, are present in the water fraction after equlibrium, and therefore have a higher affinity for water than toluene. The hydrocarbon standard seems to partition itself almost evenly between the water and the toluene fraction. It must be noted however that there are some limitations to this technique due to the fact that the tritiated standards used are generally mixtures of compounds. 50% extraction into one medium could for example represent 100% extraction of one of the compounds and 0% of another.

The hexane and DCM partition results are derived from the activity levels remaining in the aqueous phase. Once again, the hydrocarbon standard appears to partition fairly evenly between the two immiscible liquids in both the hexane and DCM experiment. The 5 other standards show close to 100% extraction into DCM, the most polar of the solvents used, and little extraction into hexane, the most non-polar solvent used.

6.6 Conclusions

Some insight into how tritiated organics may react in nature (Cardiff Bay) has been gained from the results of this chapter. The sea water/ sediment partitioning experiment, although showing little consistency, and a large range in the K_D values from ~ 1000 for the hydrocarbons and ~0 for the other standards, illustrates that the standards generally have a higher affinity for sediments in suspension, possibly due to the larger surface area of this kind of sediment.

Previous chapters have indicated that OBT present in the Cardiff Bay area has a high affinity for fine, organic rich, clay sedimentary material. Clays representative of the area, namely kaolinite and montmorillonite were employed in a series of partitioning experiments. Again the hydrocarbon standard showed the greatest affinity for the clays, with a notable preference for montmorillonite. The other standards did not show a high affinity for the clays used. This experiment does however have some limitations in that, the results could illustrate that the standards remain in one medium, due to the fact that they are hydrophobic or hydrophilic. For example the apparent high affinity for the hydrocarbon standard for montmorrilonite could actually be due to the fact that the standard is hydrophobic in nature, and that it has poor solubility in water as opposed to a high affinity for the clay surface.

With the exception of the tritiated hydrocarbons, OBT compounds were poorly extracted from water into hexane and toluene. High extraction efficiencies were however obtained with DCM. The polarity of the solvents increased from hexane, through to toluene with DCM as the most polar. The hydrocarbons present in the OBT standard are aromatic, essentially non-polar and hydrophobic in nature and should be extractible into any of the solvents investigated. The amino acids did not partition well into the non-polar solvents, due to their charged nature and consequent affinity for the aqueous phase. The majority of the OBT compounds used were polar, hydrophilic compounds and hence would not be expected to partition well into the non-polar solvents. With those compounds containing larger non-polar regions such as thymidine and nucleotides, there was some evidence of extraction. DCM is known to be a good overall solvent capable of extracting both non-polar and moderately polar species from water and this was illustrated by the extraction of OBT compounds into the DCM fraction.

The results of this chapter suggest that the compounds discharged from the Nycomed-Amersham site, and thus the tritium found in the Cardiff Bay area has a high affinity for sediments in suspension and for clay rich sediments, especially montmorrilonite.
It must be noted that there are some limitations to the experiments carried out in this chapter. The standards used to represent the Amersham-Nycomed waste released into the Cardiff Bay are in fact mixtures of compounds. The true behaviour of the individual compounds cannot be measured, and there may be instances when one compound or a group of compounds does not partition substantially into a medium, but is not measured as the results are representative of the mixture as a whole. Although the standards are supposedly representative of the waste discharged from Nycomed- Amersham, in order to have a more accurate standard for comparison purposes a mixture from the sewer outlet pipes would have been more useful. Unfortunately this was not available. The waste discharged needs to be examined more thoroughly in order to determine what compounds are discharged and which ones are likely to persist in the marine environment. At the moment the waste discharge is fairly uncharacterized.

6.7 References

Chamley, H., Clay sedimentology, Springer-Verlag eds., 1989.

Pytkowitcz, R.M., Equilibria, Nonequilibria and Natural Waters, Vol. 1, John Wiley & Sons, Publ. Inc. 1983.

7. Solvent extraction of sediment samples

7.1 Introduction

By establishing conditions in which OBT can be extracted from sediments it should be possible to deduce some of the chemical characteristics of the OBT and to enrich OBT in solution for subsequent investigation.This investigation was carried out by the successive extraction of samples with a series of solvents of increasing polarity. The solvents used for the initial extractions were (in order of increasing polarity): hexane, toluene, DCM, acetone and methanol. Soxhlet extractions were subsequently carried out using toluene, DCM and methanol. The sediments were dried with anhydrous sodium sulfate (Na_2SO_4) prior to the extraction.

7.1.1 Methodology

The initial extractions used 10g of wet sediment obtained from the same Peterstone sample, (Peterstone 15/06/00). A known mass of sediment was ground in an agate pestle and mortar with 20g of anhydrous sodium sulfate. The dried sediment was then added to a stoppered boiling tube and 20ml of solvent was added. The solution was mixed using an ultrasonic bath for 25 minutes. After settling, the solvent was then pipetted onto 5g of silica gel in an alumina boat and left overnight in a continuously pumped vacuum desiccator to remove residual solvent. The silica sample was then analysed for T using the combustion technique described in Section 3.3.3 followed by scintillation counting. A blank silica sample was also analysed to ensure that no activity was present in the silica itself.

7.1.2 Results and Discussion

Solvent extractions were carried out, using a range of extraction solvents on the same Peterstone sample (Peterstone 15/06/00) in order to try and deduce the chemical characteristics of the OBT present in the sample. The results, illustrated in Table 7.1, are shown as Bq/g of sediment analysed.

Solvent	*Date*	*Activity* *Bq/g*	*Error* *(2SD)*
hexane	17/02/01	<0.018	0.015
DCM	17/02/01	0.005	0.016
methanol	17/02/01	<0.017	0.015
acetone	17/02/01	<0.017	0.016
acetone	06/03/02	<0.017	0.014
hexane	06/03/02	<0.017	0.013
toluene	06/03/02	<0.016	0.013
DCM	06/03/02	0.016	0.015

Table 7.1: Solvent extractions of Peterstone sample 15/06/00

The results (Table 7.1) show that the OBT activity measured in the samples were below or near to the limit of detection, with the limit of detection being 0.01-0.03 Bq/g (established in Section 3.4.6).

7.1.3 Conclusions

As the activity of the sediment sample used was ~0.5 Bq/g, very little activity was extracted into any of the solvents. Even with DCM the activities were very near to the limit of detection and represented the extraction of only 1-3% of the activity in the sediment sample.

7.2 Soxhlet extractions of sediments

7.2.1 Introduction

In an attempt to improve the recovery of OBT from the sediment, Soxhlet extractions were carried out. The three solvents chosen for these extractions were toluene, DCM and methanol to cover the extraction of non-polar, semi-polar and polar OBT compounds.

7.2.2 Methodology

Approximately 5g of sediment (Peterstone 15/06/00), was weighed and added to an agate pestle and mortar and ground with 10 g of anhydrous sodium sulfate. The grainy, sand-like product was then added to a Soxhlet thimble. The sample was then Soxhlet extracted for 2 hours with 200ml of solvent. The solvent went a golden, straw-like colour with toluene and methanol; slightly less colouration was visible with DCM. Approximately 0.5-1g of silica gel was added to the extract in the round-bottomed flask, and the solvent was removed by means of rotary evaporation. Several washings with solvent were used to remove the silica to a smaller round-bottomed flask. The silica was then left overnight, under a continuously pumped vacuum, to remove any excess solvent.

Due to the possible presence residual of solvent, amendments were made to the combustion stage of the analysis procedure. These amendments were done to ensure the complete removal of the solvent, the solvents used were highly flammable and were a possible source of explosive ignition within the furnace. The silica samples were added to an alumina boat, and placed in the low-temperature furnace set at 25°C. The high temperature furnace was set at 200°C. The tube was then capped with a steady flow of air going through the tube. The high temperature furnace was then ramped to 700°C, before the ramp was set for the low temperature furnace. Oxygen was only added when the temperature was at 200°C. The water fractions were collected from the bubblers, and measured using liquid scintillation counting.

7.2.3 Results and Discussion

A series of Soxhlet extractions as carried out in order to try to better understand the characteristics of the OBT found in Cardiff Bay. The results are shown in Table 7.2.

Solvent	*Date*	*Recovery Bq/g*	*Error (2SD)*
methanol	28/03/01	0.160	0.033
methanol	01/05/01	0.055	0.022
DCM	17/04/01	0.017	0.019
DCM	17/04/01	0.028	0.020
DCM	17/04/01	0.034	0.015
toluene	28/03/01	0.070	0.029
toluene	01/05/01	0.024	0.025

Table 7.2: Soxhlet extraction data for Peterstone sample 15/06/00

The results of the soxhlet extraction, illustrate that little OBT was extracted into the solvents. The OBT activity of the sample used was ~ 0.5 Bq/g and the recoveries shown in the table above range from ~3 % recovery to 32% for the methanol extraction. The amount of activity extracted using this method was greater than the activity recovered using the other extraction procedure, yet most of the results are still close to the limit of detection (0.01-0.03 Bq/g) and variable. They cannot be interpreted as accurate extraction values.

7.3 Sediment samples

In order to verify that the OBT activity in the Peterstone sample (15/06/00), was not lost during the extraction procedure, the sediment sample left after the extraction was combusted in the furnace and its activity measured using liquid scintillation techniques. This was done to ensure that the OBT activity still remained on the sediment sample. The sediment was left in the thimble, and placed in a dessicator under a continuously pumped vaccum overnight to allow any residual solvent to evaporate. The sediment was then added to an alumina boat and combusted in the furnace using the revised method employed for the other Soxhlet extraction samples.

7.3.1 Results and Discussion

The sediment samples left after the Soxhlet extraction procedure were combusted in order to determine any potential loss of activity. The results are shown in Table 7.3.

Sample	*Date*	*Recovery* Bq/g	*Error* (2SD)
toluene/sediment	01/05/01	0.536	0.041
methanol/sediment	01/05/02	0.462	0.046
Peterstone	15/06/00	0.500	0.019

Table 7.3: Sediment samples run after solvent extraction

The results shown in Table 7.3, illustrate that even after Soxhlet extraction with varying solvents, the OBT activity appears to remain on the sediment (shown by the high values of activity remaining on the sediment). A Peterstone (15/06/00) fresh sample was also run with this experiment as a standard. The toluene/sediment sample (sediment remaining after toluene soxhlet extraction) and the methanol/sediment sample indicate no loss of activity compared to the Peterstone sample, and are both within error of that measurement. The slight variations in activity could be down to sample inhomogenaity, however all three measurements are within error of each other and hence it can be assumed that no activity was lost from the sample during the Soxhlet extraction.

7.4 Conclusions

The OBT in the sediment sample was difficult to extract, as the values shown in Tables 7.2 and 7.3 demonstrate. The highest values were found in the methanol fraction, hence it can be assumed that this fraction of the OBT was predominantly associated with polar organic compounds or inorganics co-entering into the methanol. The amount of T extracted however was small, and the sediment samples counted following the extraction verify that the activity remained on the sediment and was not extracted, or lost during the extraction.

If the OBT present on the sediments was representative of the standards supplied from Nycomed-Amersham than one would expect that, with the range of solvents employed, there would be some notable extraction. The poly-aromatic hydrocarbons, as well as the smaller molecular compounds representative of the discharge should extract in the range of polarities employed. Failure of the range of solvents to extract the OBT from the sediments therefore, suggests that it is unlikely that the OBT is in the same form as it is when discharged, and that it may be present in the cellular components of organisms or as large metabolically incorporated molecules such as protein or DNA which are not broken during extraction, or alternatively that it is strongly incorporated onto the sediment. To release the activity may require more aggressive treatments such as enzyme digests, acid digests, liquid nitrogen or a French press prior to the extraction.

Cefas (C0755), carried out Soxhlet extractions of fish caught in the Cardiff trawling ground. They also found that OBT was predominantly associated with polar organic compounds since most of the tritium in their experiments was extracted with methanol rather than hexane, and near to nothing was extracted into the DCM fraction. They found that 70% of the activity in the fish was not extracted using the three solvents, and that < 5% was extracted with DCM. There may however be an alternative reason why little OBT was extracted into the solvents. Whitehead and Breger [1950] found that organic solvents failed to extract appreciable quantities of organic material from marine sediments unless they were extensively extracted with water first. Only then did the organic solvents affect the sediment, dissolving an amazingly large amount of soluble material. Solution and removal of the salt from the sediment led to peptisation of the organic matter, making it more readily available to the organic extractant. Generally, adding a salt to a water/organic mixture leads to the precipitation of organics by destabilization of the charged groups in an aqueous/organic solvent. This process is known as 'salting out'. Sodium chloride, found extensively on the sediments as they are of marine origin, has a low effect on the solubility of organic compounds, and often reacts unfavourably with the surface of the solvent. This leads to a decrease in the solubility of the organic (OBT), a decrease in unfolding of the molecule and a minimum surface area is exposed to the solvent.

7.5 Acid-digestion of Sediment Samples

7.5.1 Introduction

A trial acid-digest experiment was carried out in order to examine the effect of acid leaching on sediment samples taken from Cardiff Bay. This was done in order to observe if there was any increase in OBT recovery compared to the extraction experiments. As this was only a trial experiment duplicates were not carried out and therefore there is an element of uncertainty in the results.

7.5.2 Methodology

10ml of conc. sulfuric acid was added to approximately 1g of Cardiff Bay sediment in a 50ml beaker. The mixture was heated on a hot plate for 30 minutes, filtered and made up to 50ml with RO water. The solution was added to a dog-bowl shaped piece of glassware [Warwick, P. et al 1999], covered with a lid and left on a hot plate for an hour, or until the centre compartment was half-full. The glassware is designed so that the water fraction evaporates and the distillate is collected in the center of the bowl. An 8ml aliquot of the collected water was taken and used for scintillation counting.

7.5.3 Results and Discussion

Two samples collected from Cardiff Bay were chosen and analysed using the acid- digestion method in order to measure the amount of OBT released. The activity of the sample as determined from the combustion technique is illustrated for comparison purposes. The results are illustrated in Table 7.4.

Sample	*Activity Bg/g*	*Error (2SD)*	*% Recovery*
Peterstone (digest)	0.410	0.287	56.72
Peterstone	0.753	0.020	
Orcherd Ledges NE (digest)	0.326	0.179	55.35
Orcherd Ledges	0.589	0.025	

Table 7.4: **Acid-digest experiment with Cardiff Bay samples.**

The results shown in Table 7.4 illustrate that with an acid-digest experiment using concentrated sulfuric acid, the OBT activity recovered was near to 50%. The results do not show very good recovery and stronger acids, such as hydrofluoric acid or chromic acid may be needed to make the digest more successful. Unfortunately, the type of reagent that can be used for the digest is limited as a result of the nature of scintillation counting techniques and the fact that halogens interfere with the scintillant and reduce its efficiency.

7.8 Summary

The various extraction methods in this chapter have produced inconclusive results which suggest that further work needs to be carried out to determine the nature and characteristics of OBT in Cardiff Bay. Although the results failed to provide further clues on the nature of the OBT found in the sediments, they were inline with those from Cefas (C0755) who also found that 70% of the tritium activity was not extracted using soxhlet extractions with DCM, methanol and hexane. The extractions also suggest that the tritium in the sediments is in a different form to that in the discharge. Washing the sediments with water first as Whitehead and Breger [1950] suggest to remove the salt, which may deter extraction, might improve the effectiveness of the techniques employed in this study. The acid/ digestion technique was tried as an alternative to soxhlet extractions, although fair tritium recoveries were obtained the method as it stands is not a viable option and does not help to characterize the nature of the tritium present in the sample.

7.7 References

CEFAS report C0755, Tritium in seafood in the Cardiff trawling ground, for Nycomed-Amersham plc. (Commercial in confidence)

Warwick, P.E., Croudace, I.W., Howard, A.G., *Improved technique for the routine determination of tritiated water in aqueous samples,* Analytica Chimica Acta, **382**, p.225-231, 1999.

Whitehead, W.L., Breger, I.A., *The origin of petroleum: effects of low-temperature pyrolysis on the organic extract of a recent marine sediment,* Science, **3**, p. 335-337, 1950.

8. Overall Conclusions

8.1 The importance of measuring OBT in the Marine Environment

The presence of bioavailable tritiated organic compounds in the waste discharged into Cardiff Bay from the Nycomed-Amersham plant has led to fish and other benthic organisms in the area, accumulating significantly higher ^3H concentrations than would be expected of fish feeding in areas subject to the discharge of tritiated water. [McCubbin et al 2001] The extent of the bioaccumulation has caused some concern over the transfer of tritium through the foodchain. It should be noted however, that whilst the ^3H concentrations in seafood found in the area are elevated, at present, such levels are considered to be of low radiological significance. [McCubbin et al 2001]

The present study was carried out in order to better understand how OBT disperses and accumulates in the marine environment at higher levels, as it could pose a threat as it transfers through the food chain into humans. Little is currently known about the metabolism of OBT in humans. Once in the body it can be incorporated into a variety of biochemical compounds such as amino acids, sugars, proteins and other structural materials.[Saito and Ishida 1989] With such a diversified array of biochemical compounds associated with OBT and each having unique metabolic processes associated with it, uniform distribution or retention of OBT compounds cannot be assumed. When dose assessments for tritium exposures are made, the fact that OBT can be directly incorporated through foodstuffs has generally been ignored since little was known about this exposure pathway for tritium. There have however been reports that the ingestion of OBT may result in 4-23 times more tritium being taken in than would be the case by inhalation, and skin absorption of HTO. [Hill and Thompson 1993, Gulden and Raskob 1992] This highlights the importance of the present study in understanding how OBT accumulates and disperses in the environment and transfers through the food chain. The distribution of OBT in the Cardiff Bay area is very much dependent upon the composition of the mixture of organically labelled compounds released in the effluent discharged from Nycomed-Amersham.

8.2 Chemical characteristics and dispersion of OBT in Cardiff Bay

For the purpose of this study, organically bound tritium (OBT) is operationally defined as the difference between total tritium and water extractable tritium measurements. This definition therefore includes all tritium compounds that remain associated with the material once the water has been physically removed.

Water extractable tritium is generally labile or exchangeable; the ^3H is bound to an electronegative atom and is thus readily exchangeable with the hydrogen atoms of water and other polar solvents. It must be borne in mind however, that water extractable tritium may also include compounds that would dissolve in water, but in which the tritium is covalently bound to a carbon atom and therefore is non-exchangeable, such as CH_2TOH. This tritium fraction therefore includes all compounds that can be extracted with or into water. Total tritium includes all the tritium in a sample, whether it be leachable in water or only released during decomposition, combustion or in the presence of strong acids, bases or catalysts.

The water-extractable tritium measurements on sediment samples collected during this study revealed that the amount of tritium leachable in water was below the limit of detection 0.02-0.04 Bq/g and thus the OBT found on the sediment was non-exchangeable and stable in nature. The non-exchangeable nature of the tritium was further illustrated by its lack of extraction into solvents with a range of polarities and poor release (~50%) during the acid digestion.The distribution of sediment-bound (non-water extractable) OBT in Cardiff Bay showed elevated levels close to the sewage discharge site in the Maerdy farm - Peterstone area. This is near the discharge point for tritium waste from the Nycomed-Amersham plant. Dispersion of the material from the sewage pipe is reflected in a drop off of sediment OBT at sites furthest away on either side, namely Goldcliff and Barry Island. Goldcliff's low levels may also be due to the fact that it has the greatest exposure to wind and wave dominant directions, and hence increased erosion. [Allen & Duffy 1998]

In addition to the spatial variability it was found that OBT levels in the sediments declined during the winter months from October to February. There are a number of possible explanations for such behaviour. Firstly, there is a significant alteration in the quantity and nature of the sediments at each site during the winter. This is caused by the increased mobility of sediments due to increased storm and wind/wave activity removing the finer particulates with which the OBT is believed to be associated. Cohen and Kniep [1973] found that the exchange and retention capability of sediments for tritium (in the form of OBT) was related to the content of organic matter and exposed hydroxyl groups of clay minerals. The Severn estuary is dominated by clay-mineral and fine grained deposits, with the Peterstone area having the finest deposits and silty clays. [Allen 1987] Obrien et al [2000] also found that on the northern shore of the Severn estuary, on the intertidal mudflats that extend between the river Rhymney at Cardiff and the river Usk at Newport, (Maerdy Farm-St Brides) the mud/sediments are strongly affected by short-term weather patterns. Secondly, biological activity is believed to decrease during these colder months reducing the quantity of OBT which might be biologically incorporated into the sediments by bacterial activity. Finally the temporal variation may reflect an alteration in the OBT discharge values or chemical composition during the year. This presumption is however not supported by any data from Nycomed-Amersham. Suggestions from McCubbin et al [2001] that the ^{3}H profile in the Cardiff area was probably as a result of short-term deposition of contaminated material from the water column onto the sediment, and that measurements of activity in the sediment samples may just simply reflect the movement of a labelled surface layer of mobile mud support all of these possible explanations for the variations in activity.

There remains some uncertainty about the nature of the OBT in the sediments found in Cardiff Bay and to the mechanisms involved in its incorporation. It is evident that the OBT is of a non-extractable (in water) and non-exchangeable nature and that it is associated with the fine grained, particulate matter suspended in the water column, but it's exact nature and composition remains unclear. CEFAS (C0984) carried out some experiments with sea water and effluent from the Amersham- Nycomed site. They found significant discrepancies between the proportion of dissolved OBT in seawater (~3-4%) and a spot effluent sample they examined (~30-55%). This might indicate that there is degradation of the OBT compounds along the passage through the Cardiff sewer system or upon release into the Bristol Channel. Another possible explanation is that there is sorption onto particulate material whilst in the sewer system, or more rapid sorption in the Bristol Channel. Results from one of their laboratory experiments confirms that there is rapid uptake by suspended particulate material, together with a process conversion of OBT to HTO with time. CEFAS (C0984) concluded that T uptake occurred presumably as a result of OBT binding to the film of natural surface-active organic matter, which dominates the surface chemistry of solid surfaces in sea water. [Hunter 1980] In turn, McCubbin et al [2001], noted that there might be several factors affecting the accumulation of ^3H by particulate material including: physio-chemical sorption, bacterial uptake in seawater, bacterial uptake in the Cardiff sewer prior to mixing of the effluent with seawater. Physico-chemical sorption could be due to binding of dissolved ^3H-organic compounds to the film of natural surface-active organic matter, or as a result of the association of tritium bearing bacteria with suspended particles or even as a result of the accumulation by sedimentary material. Joint and Pomroy [1982] showed through a study of the microbial behaviour in the Bristol Channel that an estimated ~ 50% of bacteria are attached to suspended particles. These findings produce a wide range of possibilities for the fact that tritium is found in sediment samples in the area, whether the activity is in fact associated with the sediment itself, as a result of physico-chemical sorption by the sedimentary material, or whether it is in fact found in organisms associated with particulate material, which have subsequently attached themselves onto the sediments remains unknown. The failure of solvents to extract OBT from sediments analysed in this study suggest that the OBT bound to the sediment is not characteritic of the compounds discharged and that it is strongly incorporated on to the sediment, with more than just an absorption mechanism. It may be present in cellular structures or it could be biologically incorporated into large molecules such as DNA or proteins, which are not extractable using solvents. Further, more rigorous techniques need to be employed that would allow the breaking of cellular structures by enzymatic or chemical cleavage thus releasing the OBT in a more extractable form. The study would suggest however, that the activity is present in the particulate material, whether associated with organisms or not, which is constantly deposited and re-deposited on the sediments and acts therefore as a mobile layer which covers the underlying sediment but is not in fact associated with it.

8.3 Further work

Further work needs to be carried out in order to establish what form of tritium (OBT) is present in the samples in the Cardiff Bay area. Until more is known about the characteristics and nature of the tritium found in the sediments, predictions on its residence time and on the effects it might have on the environment are hard to make. Further work needs to be carried out on the effluent, and how the compounds interact with the organisms found in the sewer system to determine whether the tritium is already incorporated into organisms before it reaches the Bay, and how the numerous compounds interact with each other.

From the present study, it has been concluded that the OBT found in Cardiff Bay is associated with fine, organic rich particulate matter or organisms in the water column. Basic extraction and digestion experiments have been carried out highlighting the possibility that the OBT is no longer in the same form as it was when discharged, which may suggest that the OBT is within sediment organisms such as algae and bacteria. In order to release the tritium from the organisms stronger digestion processes may therefore be required, this may be achieved using alternative reagents such as chromic acid or a French press.

There are a number of ongoing studies investigating the release of OBT into the aquatic environment and the impacts of its discharge. Substantial further work needs to be carried out before the 'true' impacts of this discharge and the subsequent effects are revealed and properly understood.

8.4 References

Allen, J.R.L., Reworking of muddy intertidal sediments in the Severn Estuary, Southwestern U.K; a preliminary survey, Sedimentary Geology, 50,p.1-23, 1987.

Allen, J.R.L., Duffy, M.J., *Medium-term sedimentation on high intertidal mudflats and salt marshes in the Severn Estuary, SW Britain: the role of wind and tide,* Marine Geology, **150**, p. 1-27, Sep. 1998.

CEFAS Contact tender C0984, *An assessment of the transfer of tritium through the marine foodchain,* For Nycomed-Amersham, commercial in confidence.

Cohen, L.K. and Kniep, T.J., *Environmental tritium studies at a PWR plant,* In: Tritium. Eds. A.A. Moghissi and M.W. Carter (Phoenix, AZ: messenger Graphics) p.623-639, 1973.

Gulden, W., Raskob, W., *Accidental tritium doses based on realistic modelling,* Fusion Technology, **21**, pp. 536-643, 1992.

Hill, R.J., Thompson, J.R., *Metabolism and dosimetry of tritium,* Health Physics, **65**, pp.628-647, 1993.

Hunter, K.A., *Microelectrophoretic properties of natural surface-active organic matter in coastal seawater,* Limnology and Oceanography, **25**, pp. 807-822, 1980.

Joint, I.R., Pomroy, A.J., *Aspects of microbial heterotrophic production in a highly turbid estuary,* Journal of Experimental Marine Biology and Ecology, **58**, pp.33-46, 1982.

McCubbin, D., Leonard, K.S., Bailey, T.A., Williams, J., *Incorporation of organic tritium (^{3}H) by marine organisms and sediment in the Severn Estuary/Bristol Channel (UK),* Marine Pollution Bulletin, **42**, pp. 852-863, 2001.

Obrien, D.J., Whitehouse, R.J.S., Cramp, A., *The cyclic development of a macrotidal mudflat on varying timescales.* Continental Shelf Research, **20**, p.1593-1619, 2000.

Saito, M., Ishida, M.R., *Dose-modification factor for accumulated dose to cell nucleus due to protein-bound* ^{3}H, Health Physics, **56**, pp.869-874, 1989.

9. Appendix 1 – Sampling Sites

Site Name: 1 (Barry Island)

Dates sampled: 24/02/00, 21/03/00,17/05/00, 15/06/00, 31/07/00, 25/09/00, 25/10/00, 08/12/00, 06/02/01.

Location: Barry Harbour, due west of Barry island, at the mouth of the Severn Estuary, just off the car park for the 'Pleasure Park'.

Grid Ref: ST 10972 66542

Site description: Harbour inlet with a narrow ~50m connection to the open sea. Substrate mainly silty sand, overleign by a surface layer of mud. The soft surface mud is over a denser/darker (anoxic? mud). Samples taken from an area of fine mud. In winter the mud was 1-2cm thick, layer increased to 10-15cm during spring. There is local colonisation by *Spartina sp.* forming raised mud mounds, and fucoidital seaweed is present. Isolated mounds of pioneer salt marsh are also present. Marsh here is possibly expanding laterally.

Site Name: 2 (Orchard Ledges S.W.)

Dates sampled: 24/02/00, 21/03/00, 17/05/00, 15/06/00, 31/07/00, 25/09/00, 25/10/00, 08/12/00, 06/02/01.

Location: Orchard Ledges, open mudflat joining Bristol channel, seaward of Cardiff docks, due east of 'Queen Alexandra Dock', just off coastal road.

Grid Reference: ST 19973 73355

Site description: Intertidal mudflat. Substrate mud and black grit, possibly anthropogenic.Coarse sand, little fine material present. Ridges and runnels present on the flats. Site is backed by a sloping sandy beach. No seaweed. Presence of much anthropogenic debrie; bricks, concrete, slabs, smelt products, although more sparsely than the other Orchard Ledges site.

Site Name: 3 (Orchard Ledges N.E)

Dates sampled: 24/02/00, 21/03/00, 17/05/00, 15/06/00, 31/07/00, 25/09/00, 25/10/00, 08/12/00, 06/02/01.

Site location: Orchard Ledges, open mudflat joining Bristol channel, seaward of Cardiff docks. Site is north east from previous site, about 500m along the coastal road.

Grid Reference: ST 20807 74282

Site description: Intertidal mudflats consisting of mud and sand. The flats are backed by a gently sloping area of boulders, mostly derived from industrial activity (boulders are mainly concrete brick and industrial slags). Sand is black and possibly derived from exposed sections of coal and slag. *Fucus serratus* present.

Site Name: 4 (Maerdy Farm)

Dates sampled: 24/02/00, 21/03/00, 17/05/00, 15/06/00, 31/07/00, 25/09/00, 25/10/00, 08/12/00, 06/02/01.

Site location: Rumney Great Wharf area, Severn Estuary. Seaward of Maerdy Farm, just off coastal path (seaward side).

Grid Reference: ST 23199 77627

Site description: Intertidal flats, consisting of compact, eroded former marsh overlain by thin veneer of soft, fluid, clay-rich sediment. The marsh and mudflat are backed by a seawall. The erosion surface of the former saltmarsh extends 100m out into the estuary from the seawall. The thin veneer of surface sediments (5-10 cm thick) sampled probably represent material that is resuspended and deposited on each tidal cycle, overlying a fairly inert, compacted clay surface. Surface sediment eroded mainly during winter, with big declines in October and December, although a decline was also evident during June. In December there was no mud, and the eroded saltmarsh substrate was stripped bare by the storm activity. The only sediment present was coarse sand and shell debrie. There was evidence of flotsam on the seawall, probably washed in by the storms.

Site Name: 5 (Peterstone Great Wharf)

Dates sampled: 24/02/00, 21/03/00, 17/05/00, 15/06/00, 31/07/00, 25/09/00, 25/10/00, 06/02/01.

Site location: Northern shore of the Severn Estuary, seaward of the village of Peterstone Wentlooge. Site is north easterly of Maerdy farm, along Peterstone Great Wharf.

Grid Reference: ST 27025 79744

Site description: Intertidal mudflat. The intertidal area faces south-east and has a width of 1km and a slope of 1:94. It is backed by a salt marsh (approx 60m wide) and a sea defense wall. The edge of the current marsh shows ridge and runnel topography, the marsh is apparently eroding and retreating. The mudflat is protect from swell waves entering the estuary from the west but can experience events due to short period wind-waves generated within the estuary. The layer of mud varied in thickness over time, in spring it was 6-10cm thick, already by June there was evidence of erosion, by October there was only a very thin veneer visible and in December there was not enough present to collect a sample. In February there was only a very thin layer available.

Site Name: 6 (St Brides, Wentlooge)

Dates sampled: 24/02/00, 21/03/00, 17/05/00, 15/06/00, 25/09/00, 25/10/00, 08/12/00, 06/02/01.

Site location: Northern shore of the Severn Estuary, seaward of the village of St Brides Wentlooge. Just over the seawall from the 'Six Bells' public house car park.

Grid Reference: ST 30092 81536

Site description: Intertidal mudflat, with soft mud substrate. Backed by seawall with limestone block debris at base. Approximately 20 m seaward of the sampling site remains of the former marsh surface outcrops. This presumably underlies the sampling site. *Fucoid sp.* is present on boulders at the rear of the mudflat. The layer of mud varied in thickness over time, in spring it was 6-10cm thick, already by June there was evidence of erosion. By October there was only a thin layer of sample left. In December and February there was also very little present.

Site Name: 7 (Gold Cliff)

Dates sampled: 24/02/00, 21/03/00, 17/05/00, 15/06/00, 31/07/00, 25/09/00, 25/10/00, 26/02/01.

Site location: Northern shore of Severn Estuary, Gold Cliff. Over seawall from car park.

Grid Reference: ST 37495 82131

Site description: Intertidal mudflat, with soft mud substrate. Backed by seawall, the substrate is overlying compact mud and sand- possibly an eroded marsh surface. The layer of mud varied in thickness over time, in spring it was 6-10cm thick, already by June there was evidence of erosion, by October there was only a very thin veneer visible and in December there was not enough present to collect a sample. In February there was only a very thin layer available.

Appendix 2.0: Scintillant efficiency calculations

Scintillant	Aq:scint	Water type	aqueous	CPM	error	CPS	SQP (Quench)	error	error in SQP	Bkg	error	Spike mass (g)	Spike SpA Bq/g	Activity added/Bq	Eff	relative uncert	absolute uncert
Ultima Gold LLT	07:13	RO	7.00	315.54	0.73	5.26	756.95	0.16	0.002	6.24	5.2		92.14	16.68	31.53	0.02	0.67
Ultima Gold LLT	08:12	RO	8.00	287.28	0.77	4.79	741.73	0.20	0.002	5.18	5.71		92.14	17.33	27.63	0.02	0.59
Ultima Gold LLT	09:11	RO	9.00	275.73	0.78	4.60	740.19	0.15	0.002	6.36	5.15		92.14	17.59	26.12	0.02	0.56
Ultima Gold LLT	10:10	RO	10.00	266.74	0.80	4.45	719.06	0.20	0.002	4.77	5.94		92.14	17.87	24.88	0.02	0.54
Ultima Gold LLT	12:08	RO	12.00	211.06	0.89	3:52	682.18	0.17	0.002	4.06	6.44		92.14	17.74	19.82	0.02	0.43
Gold Star	02:18	RO	2.00	330.6	0.51	5.51	779.03	0.13	0.001	1.17	5.39	0.204	92.14	18.80	29.31	0.02	0.61
Gold Star	03:17	RO	3.00	444.49	0.44	7.41	773.02	0.15	0.002	0.91	5.33	0.288	92.14	26.54	27.92	0.02	0.57
Gold Star	05:15	RO	5.00	295.65	0.53	4.93	757.16	0.13	0.001	1.11	5.15	0.208	92.14	19.17	25.71	0.02	0.53
Gold Star	07:13	RO	7.00	272.26	0.79	4.54	736.47	0.15	0.002	4.33	6.24		92.14	17.92	25.31	0.02	0.54
Gold Star	08:12	RO	8.00	247.71	0.83	4.13	723.65	0.15	0.002	3.84	6.62		92.14	17.29	23.88	0.02	0.52
Gold Star	09:11	RO	9.00	221.53	0.87	3.69	714.55	0.22	0.002	3.84	6.62		92.14	17.98	20.54	0.02	0.45
Gold Star	10:10	RO	10.00	240.81	0.84	4.01	704.72	0.24	0.002	3.37	7.07		92.14	18.01	22.28	0.02	0.48
Gold Star	12:08	RO	12.00	157.78	1.03	2.63	666.08	0.22	0.002	3.45	6.98		92.14	17.81	14.77	0.02	0.33
Gold Star	01:19	Sea	1.00	329.08	1.84	5.48	666.08	0.22	0.002	2.59	1.99	0.203	92.14	17.81	30.80	0.03	0.84
Gold Star	02:18	Sea	2.00	324.07	1.16	5.40	778.33	0.16	0.002	1.55	5.44	0.201	92.14	18.52	29.16	0.02	0.67
Gold Star	05:15	Sea	5.00	285.68	0.99	4.76	755.22	0.17	0.002	2.68	4.86	0.197	92.14	18.15	26.23	0.02	0.59
Gold Star	05:15	Toluene	5.00	347.9	1.03	5.80	822.95	0.19	0.002	1.8	0.56	0.2	93.14	18.63	31.13	0.02	0.70
Picofluor LLT	07:13	RO	7.00	270.81	0.79	4.51	724.50	0.12	0.001	5.75	5.42		92.14	17.75	25.42	0.02	0.55
Picofluor LLT	08:12	RO	8.00	246.48	1.02	4.11	718.02	0.18	0.002	5.75	5.42		92.14	17.08	24.05	0.02	0.54
Picofluor LLT	09:11	RO	9.00	223.27	0.87	3.72	707.32	0.17	0.002	6.10	5.26		92.14	17.69	21.03	0.02	0.46
Picofluor LLT	10:10	RO	10.00	217.83	0.88	3.63	693.76	0.19	0.002	4.59	6.06		92.14	17.95	20.22	0.02	0.44
Picofluor LLT	12:08	RO	12.00	162.41	1.02	2.71	670.18	0.20	0.002	3.74	6.71		92.14	17.71	15.29	0.02	0.34
Optiphase 'Trisafe'	07:13	RO	7.00	303.88	0.75	5.06	755.63	0.15	0.002	4.21	6.32		92.14	17.88	28.33	0.02	0.61
Optiphase 'Trisafe'	08:12	RO	8.00	271.88	0.79	4.53	747.10	0.20	0.002				92.14	18.01	25.17	0.02	0.54
Optiphase 'Trisafe'	09:11	RO	9.00	224.72	0.87	3.75	733.29	0.31	0.003	3.59	6.9		92.14	17.90	20.93	0.02	0.46
Optiphase 'Trisafe'	10:10	RO	10.00	185.35	0.95	3.09	713.14	0.21	0.002	3.39	7.05		92.14	18.08	17.09	0.02	0.38
Optiphase 'Trisafe'	12:08	RO	12.00	119.95	1.19	2.00	678.23	0.18	0.002				92.14	17.96	11.13	0.02	0.26
Aquasafe 500 plus	07:13	RO	7.00	273.74	0.79	4.56	734.81	0.16	0.002	3.56	6.88		92.14	18.00	25.35	0.02	0.55
Aquasafe 500 plus	08:12	RO	8.00	221.13	0.87	3.69	716.38	0.24	0.002	3.79	6.67		92.14	17.81	20.70	0.02	0.45
Aquasafe 500 plus	09:11	RO	9.00	158.16	1.03	2.64	702.43	0.84	0.008	10.76	3.96		92.14	17.95	14.68	0.02	0.33
Aquasafe 500 plus	10:10	RO	10.00	113.19	1.22	1.89	725.41	1.08	0.011	11.64	3.81		92.14	18.01	10.47	0.02	0.25
Aquasafe 500 plus	12:08	RO	12.00	64.97	1.61	1.08	688.79	0.73	0.007	10.59	3.99		92.14	17.91	6.04	0.03	0.16
Aquasafe 300 plus	07:13	RO	7.00	222.44	0.87	3.71	768.04	0.24	0.002	11.12	3.9		92.14	18.03	20.56	0.02	0.45
Aquasafe 300 plus	08:12	RO	8.00	125.37	1.16	2.09	759.72	0.32	0.003	7.96	4.6		92.14	17.56	11.90	0.02	0.28
Aquasafe 300 plus	09:11	RO	9.00	114.20	1.22	1.90	762.97	0.38	0.004	8.91	4.35		92.14	17.82	10.68	0.02	0.25
Aquasafe 300 plus	10:10	RO	10.00	91.96	1.35	1.53	735.82	0.34	0.003	8.11	4.56		92.14	17.71	8.65	0.02	0.21
Aquasafe 300 plus	12:08	RO	12.00	62.34	1.65	1.04	675.89	0.38	0.004	7.72	4.67		92.14	17.87	5.81	0.03	0.15

Appendix 2.1 Reproducibility- 8ml Ro water/ 22ml Gold Star

	Date / time of count	count time (min)	SQPE (Quench)	cpm	delayed coincidence	ccpm
RO Blank 1	26/09/2000 05:41	120.00	727.49	2.56	0.99	1.57
RO Blank 1	26/09/2000 07:43	120.00	728.55	3.02	1.14	1.88
RO Blank 1	26/09/2000 09:45	120.00	729.49	3.19	0.97	2.22
RO Blank 1	26/09/2000 11:48	120.00	731.12	2.80	1.17	1.63
RO Blank 1	26/09/2000 13:50	120.00	728.27	3.03	1.11	1.92
RO Blank 2	27/09/2000 07:18	120.00	731.05	2.54	0.85	1.69
RO Blank 2	27/09/2000 09:21	120.00	729.9	2.49	1.19	1.30
RO Blank 2	27/09/2000 11:23	120.00	728.93	2.45	0.85	1.60
RO Blank 2	27/09/2000 13:25	120.00	728.98	2.44	1.14	1.30
						1.68
RO Blank	19/04/2000 12:25	60.00	728.65	4.18	3.1	1.08
RO Blank	19/04/2000 13:28	60.00	726.4	2.53	2.24	0.29
RO Blank	19/04/2000 14:31	60.00	726.21	2.78	2.16	0.62
RO Blank	19/04/2000 15:34	60.00	728.66	2.61	2.04	0.57
RO Blank	19/04/2000 16:36	60.00	727.84	2.53	1.87	0.66
RO Blank	19/04/2000 17:39	60.00	726.6	2.44	2.16	0.28
RO Blank	19/04/2000 18:42	60.00	726.15	2.59	2.12	0.47
RO Blank	19/04/2000 19:45	60.00	726.25	2.68	2.43	0.25
RO Blank	19/04/2000 20:48	60.00	723.7	2.80	2.32	0.48
RO Blank	19/04/2000 21:50	60.00	725.81	2.48	2.19	0.29
RO Blank	19/04/2000 22:53	60.00	725.86	2.83	1.84	0.99
RO Blank	19/04/2000 23:56	60.00	726.4	2.66	2.19	0.47
RO Blank	19/04/2000 00:59	60.00	723.23	2.87	2.38	0.49
RO Blank	19/04/2000 02:02	60.00	727.52	3.32	2.11	1.21
RO Blank	19/04/2000 03:04	60.00	724.13	3.27	2.46	0.81
RO Blank	19/04/2000 04:07	60.00	728.31	3.08	2.04	1.04
RO Blank	19/04/2000 05:10	60.00	725.56	3.24	2.19	1.05
RO Blank	19/04/2000 06:13	60.00	727.63	3.42	2.73	0.69
RO Blank	19/04/2000 07:15	60.00	724.36	3.56	2	1.56
RO Blank	19/04/2000 08:18	60.00	724.84	3.59	2.54	1.05
RO Blank	19/04/2000 09:21	60.00	724.84	3.08	2.46	0.62
RO Blank	19/04/2000 10:24	60.00	725.35	3.56	2.49	1.07
RO Blank	19/04/2000 11:27	60.00	724.67	3.32	2.71	0.61
RO Blank	19/04/2000 12:29	60.00	724.89	3.30	2.92	0.38
						0.71

Appendix 2.2: Calculations (including uncertainties) for Liquid Scintillation Couting

CPM window 1	C_1
CPM window 3	C_3 (delayed coincidence count)
Background window 1	B_1
Background window 3	B_3 (delayed coincidence count)
Counting efficiency	E (from calibration report, shown in Table 10.2)
Mass of sample analysed	M
Volume of distillate analysed (ml)	V
^{3}H half life	12.35 yrs
Elapsed time in days	t_{elapse} (between count date and reference date)
Count time (mins)	t

Corrected sample CPM (CCPM) $= C_1 - C_3 \quad = C_{corr}$

Corrected Background $= B_1 - B_3 \quad = B_{corr}$

Calculations of tritium activities

$$\mathrm{Bq} / \mathrm{g} = \frac{C_{corr} - B_{corr}}{60} \times \frac{100}{E} \times \frac{50}{V} \times \frac{1}{M} \times \exp\left(\frac{\ln 2}{12.35 \times 365.25} \times t_{elapse}\right)$$

Calculations of uncertainties (2.d counting statistical)

$$\mathrm{Bq} / \mathrm{g} = \frac{\sqrt{(C_1 + C_3 + B_1 + B_3) \times t}}{t \times 60} \times \frac{100}{E} \times \frac{50}{V} \times \frac{1}{M} \times \exp\left(\frac{\ln 2}{12.35 \times 365.25} \times t_{elapse}\right)$$